(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
19 January 2006 (19.01.2006)

PCT

(10) International Publication Number
WO 2006/005173 A1

(51) International Patent Classification[7]: A61K 31/675, 31/455, 31/216, A61P 7/00, 3/06

(21) International Application Number: PCT/CA2005/001070

(22) International Filing Date: 11 July 2005 (11.07.2005)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
60/586,214 9 July 2004 (09.07.2004) US

(71) Applicant *(for all designated States except US)*: **MEDICURE INTERNATIONAL INC.** [CA/CA]; 4 - 1200 Waverley Street, Winnipeg, Manitoba R3T 0P4 (CA).

(72) Inventors; and

(75) Inventors/Applicants *(for US only)*: **ZETTLER, Marjorie** [CA/CA]; Apt. # 1, 141 River Avenue, Winnipeg, Manitoba R3L 0A8 (CA). **KHALIL, Ahmad** [CA/CA]; 121 Gobert Crescent, Winnipeg, Manitoba R2N 2Z3 (CA).

(74) Agent: **RIDOUT & MAYBEE LLP**; One Queen Street East, Suite 2400, Toronto, Ontario M5C 3B1 (CA).

(81) Designated States *(unless otherwise indicated, for every kind of national protection available)*: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BW, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KM, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NA, NG, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, SM, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW.

(84) Designated States *(unless otherwise indicated, for every kind of regional protection available)*: ARIPO (BW, GH, GM, KE, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IS, IT, LT, LU, LV, MC, NL, PL, PT, RO, SE, SI, SK, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Published:
— *with international search report*

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

WO 2006/005173 A1

(54) Title: COMBINATION THERAPIES EMPLOYING NICOTINIC ACID DERIVATIVES OR FIBRIC ACID DERIVATIVES

(57) Abstract: The present invention provides pharmaceutical compositions comprising a nicotinic acid derivative or a fibric acid derivative and a pyridoxal-5'-phosphate or a pyridoxal-5'-phosphate related compound and methods for using the pharmaceutical compositions for reducing the risk of cardiovascular and other diseases.

COMBINATION THERAPIES EMPLOYING NICOTINIC ACID DERIVATIVES OR FIBRIC ACID DERIVATIVES

This application claims priority to US provisional patent application 60/586,214, the disclosure of which is hereby incorporated in its entirety.

FIELD OF INVENTION

This invention generally relates to combination therapies employing nicotinic acid derivatives or fibric acid derivatives, and uses thereof.

BACKGROUND

According to the American Heart Association in 2000, 39.4 % of deaths were from cardiovascular disease. The risk of developing heart disease and indirectly stroke, increases steadily as blood cholesterol values rise. Elevated blood cholesterol levels are also associated with an increased risk of developing diabetes. The desirable blood levels are < 200 mg/dL. Borderline acceptable levels are in the range of 200-239 mg/dL and high risk begins at 240 md/dL or greater. It is estimated that some 102.3 million Americans have high cholesterol numbers.

Hypercholesterolemia is known to affect the responsiveness of various blood vessels to endogenous and exogenous vasoactive agents. Of particular interest is the increased responsiveness to vasoconstrictors, e.g. 5-hydroxy tryptamine and noradrenaline, and the decreased reactivity towards vasodilators, e.g. acetylcholine and nitric oxide. This together with the development of arteriosclerosis plays an important role in the progression of many cardiovascular-related disorders, such as hypertension, stroke and coronary artery disease.

Presently hypercholesterolemia is treated primarily with lipid lowering drugs such as statins, fibrates or niacin. While these drugs are effective for lowering lipid levels, the use of these drugs, alone and in combination with other drugs, is limited due to adverse side effects and drug-drug reactions, including most significantly, the

inhibition of hepatic cytochrome P450 enzymes, which are responsible for the metabolism of drugs in the liver.

In contrast, vitamin B6 which also has lipid lowering properties, is a well tolerated drug with no significant side effects (Brattstrom et al, Pyroxidine reduces cholesterol and low-density lipoprotein and increases antithrombin III activity in 80 year old men with low plasma pyridoxal 5-phosphate, Scand J Clin Lab Invest, 1990, 50:873). Several vitamin B6 derivatives also have lipid-lowering properties. For example, US Patent No. 6,066,659 teaches the use of vitamin B6 (pyridoxine), pyridoxal and pyridoxamine derivatives for the treatment of hyperlipidemia and atherosclerosis. German Patent DE 24 61 742 C2 teaches the use of pyridoxal, pyridoxol, and pyridoxamine -5'phosphoric acid esters for treating hyperlipidemia. Supplementation with magnesium pyridoxal-5'-phosphate glutamate, has also been shown to reduce lipid levels (Khayyal et al, Effect of magnesium pyridoxal 5-phosphate glutamate on vascular reactivity in experimental hypercholesterolemia, Drugs Exp Clin Res. 1998, 24:29-40).

In addition to lipid lowering properties, vitamin B6 and its metabolites, such as pyridoxal-5'-phosphate, are useful in the treatment of cardiovascular or related disease, for example, myocardial ischemia and ischemia reperfusion injury, myocardial infarction, cardiac hypertrophy, hypertension, congestive heart failure, heart failure subsequent to myocardial infarction, vascular disease including atheroclerosis, and diseases that arise from thrombotic and prothrombotic states in which the coagulation cascade is activated.

Previous disclosures have taught the optional use of vitamin B6 (pyroxidine) with a cholesterol-lowering agent wherein the inclusion of vitamin B6 was directed to decreasing homocysteine levels. For example, US Patent 6,576,256 discloses a method of treating a patient with elevated cardiovascular risk by the use of a HMG CoA reductase inhibitor with an inhibitor for the renin-angiotension system, aspirin and optionally vitamin B6 (pyridoxine). US Patent Application No. 20030049314

discloses a formulation for treating a patient with elevated cardiovascular risk comprising a combination of an HMG Co A reductase inhibitor, an ACE inhibitor, aspirin and optionally vitamin B6. US Patent Application No. 20030068399 discloses an orally administrable pharmaceutical dosage form for treating a patient at elevated cardiovascular risk comprising a combination of an HMG Co A reductase inhibitor, an inhibitor for the renin-angiotension system, aspirin and optionally vitamin B6. US Patent No. 6,669,955 discloses an orally administrable pharmaceutical dosage form for reducing the risk of a cardiovascular event, comprising a combination of fibric acid derivative, an inhibitor for the renin-angiotension system, aspirin and optionally vitamin B6. However, there are currently no combination therapies which employ a pyridoxal-5'-phosphate or pyridoxal-5'-phosphate related compound as a lipid-lowering agent in combination with another class of lipid lowering agents, and in particular niacin or fibrates.

The use of nicotinic acid derivatives (such as niacin) or fibric acid derivatives (fibrates) in combination with other drugs, and consequently the potential for additive therapeutic benefits, has been limited because of hepatotoxicity. There are currently no combination therapies for treating and preventing hypercholesterolemia and related disorders such as cardiovascular disease and diabetes which do not induce adverse drug reactions and which are suitable for persons susceptible to drug-induced hepatotoxicity. Accordingly, there is a need for new pharmaceutical compositions and methods of treatment which overcome the limitations of the current therapies involving nicotinic acid derivatives or fibric acid derivatives.

SUMMARY OF INVENTION

The present invention provides a pharmaceutical composition comprising: (a) a nicotinic acid derivative or a fibric acid derivative; (b) pyridoxal-5'-phosphate or a pyridoxal-5'-phosphate related compound; and (c) a pharmaceutically acceptable carrier.

In one embodiment of the invention, the fibric acid derivative is selected from a group consisting of: bezafibrate, clofibrate, ciprofibrate, fenofibrate, gemfibrozil, and a mixture thereof.

In another embodiment of the invention, the nicotinic acid derivative is selected from a group consisting of niacin, niceritol, acipimox and acifran.

In another embodiment of the invention, the pyridoxal-5'-phosphate related compound is selected from a group consisting: pyridoxal, pyridoxal-5'-phosphate, pyridoxamine, a 3-acylated analogue of pyridoxal, a 3-acylated analogue of pyridoxal-4,5-aminal, a pyridoxine phosphate analogue, and a mixture thereof.

The present invention also provides a method for treating a patient at risk of cardiovascular disease comprising administering a therapeutically effective dose of the pharmaceutical composition comprising: (a) a nicotinic acid derivative or a fibric acid derivative; (b) a pyridoxal-5'-phosphate or pyridoxal-5'-phosphate related compound; and (c) a pharmaceutically acceptable carrier.

In an embodiment, the method is for treating the patient susceptible to hepatotoxicity.

The cardiovascular disease may be selected from a group consisting: congestive heart failure, myocardial ischemia, arrhythmia, myocardial infarction, ischemic stroke, hemorrhagic stroke, coronary artery disease, hypertension (high blood pressure), atherosclerosis (clogging of the arteries), aneurysm, peripheral artery disease (PAD), thrombophlebitis (vein inflammation), diseases of the heart lining, diseases of the heart muscle, carditis, congestive heart failure, endocarditis, ischemic heart disease, valvular heart disease (malfunction of a valve or valves in the blood vessels of the heart), arteriosclerosis (hardening of the arteries), acute coronary syndrome (ACS), deep vein thrombosis (DVT), Kawazaki disease, and heart transplant.

The present invention also provides a method for treating a patient at risk of diabetes comprising administering a therapeutically effective dose of the pharmaceutical composition comprising: (a) a nicotinic acid derivative or a fibric acid derivative; (b) a pyridoxal-5'-phosphate or pyridoxal-5'-phosphate related compound; and (c) a pharmaceutically acceptable carrier.

The dose of the nicotinic acid derivative may be between 0.1 and 5000 mg per day. The dose may be between 100 and 3000 mg per day. The dose may be 100, 250, 500, 1000, or 3000 mg per day.

The dose of the fibric acid derivative may be between 0.1 and 1000 mg per day. The dose may be between 43 and 200 mg per day. The dose may be between 160 and 200 mg per day. The dose may be 100, 200, 400, or 600 mg per day.

The dose of the pyridoxal-5'-phosphate or pyridoxal-5'-phosphate related compound may be between 0.1 to 50 mg/kg per day. The dose of pyridoxal-5'-phosphate or pyridoxal-5'-phosphate related compound may be between 1 to 15 mg/kg per day.

The present invention further provides a method of treating or preventing hypercholesterolemia in a patient comprising administering a therapeutically effective dose of: (a) a nicotinic acid derivative or a fibric acid derivative and (b) a pyridoxal-5'-phosphate or pyridoxal-5'-phosphate related compound wherein the pyridoxal-5'-phosphate related compound is selected from a group consisting: pyridoxal-5'-phosphate, a 3-acylated analogue of pyridoxal, a 3-acylated analogue of pyridoxal-4,5-aminal, a pyridoxine phosphate analogue, and a mixture thereof.

The present invention further provides the use of a pyridoxal-5'-phosphate or pyridoxal-5'-phosphate related compound to decrease the side effects of nicotinic acid derivative administration. The nicotinic acid derivative may be niacin; the side effect may be an elevated homocysteine level and/or an elevated thromboxane A2 level.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 shows the effect of pyridoxal 5'-phosphate on the fluorescence of the various metabolic products measured in the CYP inhibition assays. Figures 1(a) to 1(f), illustrate the decrease in the fluorescence of the metabolic products (CHC, 7-HC, HFC, fluorescein, AHMC and quinolinol) measured in the CYP inhibition assays as a function of pyridoxal 5'-phosphate concentration.

Figures 2(a) and 2(b) illustrate the inhibition of the catalytic activity of CYP1A2 (metabolism of CEC to CHC) as a function of Furafylline and P5P concentration respectively.

Figures 3(a) and 3(b) illustrate the inhibition of the catalytic activity of CYP2A6 (metabolism of coumarin to 7-HC) as a function of Tranylcypromine and P5P concentration respectively.

Figures 4(a) and 4(b) illustrate the inhibition of the catalytic activity of CYP2B6 (metabolism of EFC to HFC) as a function of Tranylcypromine and P5P concentration respectively.

Figures 5(a) and 5(b) illustrate the inhibition of the catalytic activity of CYP2C8 (metabolism of DBF to Fluorescein) as a function of Quercetin and P5P concentration respectively.

Figures 6(a) and 6(b) illustrate the inhibition of the catalytic activity of CYP2C9 (metabolism of MFC to HFC) as a function of Sulfaphenazole and P5P concentration respectively.

Figures 7(a) and 7(b) illustrate the inhibition of catalytic activity of CYP2C19 (metabolism of CEC to CHC) as a function of Tranylcypromine and P5P concentration respectively.

Figures 8(a) and 8(b) illustrate the inhibition of the catalytic activity of CYP2D6 (metabolism of AMMC to AHMC) as a function of Quinidine and P5P concentration respectively.

Figures graphs 9(a) and 9(b) illustrate the inhibition of the catalytic activity of CYP2E1 (metabolism of MFC to HFC) as a function of Diethyldithiocarbamic acid (DDTC) and P5P concentration respectively.

Figures 10(a) and 10(b) illustrate the inhibition of the catalytic activity of CYP3A4 (metabolism of BFC to HFC) as a function of Ketoconazole and P5P concentration respectively.

Figures 11(a) and 11(b) illustrate the inhibition of the catalytic activity of CYP3A4 (metabolism of BQ to Quinolinol) as a function of Ketoconazole and P5P concentration.

Figure 12 summarizes the IC_{50} values estimated for the known inhibitors of each CYP subtype, and for pyridoxal 5'-phosphate.

Figure 13 illustrates the area under the curve CK-MB values fitted to a log-normal distribution for patients treated with P5P (A) and placebo (B).

Figure 14 (Table 1) summarizes baseline clinical, electrocardiographic, and angiographic characteristics in patients treated with P5P or placebo.

Figure 15 (Table 2) summarizes procedural and angiographic results for patients treated with P5P or placebo.

Figure 16 (Table 3) summarizes periprocedural cardiac markers and ST monitoring results for patients treated with P5P or placebo.

DETAILED DESCRIPTION OF THE INVENTION

The causal association between elevated low density lipoproteins (LDL) levels and the risk for developing cardiovascular disease is well established. Reducing elevated LDL levels have been shown to reduce the incidence of cardiovascular events, including transient ischemic attacks and indirectly strokes, and to reduce mortality. More recently, elevated LDL levels have been correlated with an increased risk for developing diabetes.

Niacin has been used to lower the risk of heart disease. While the mechanism of action is not yet clear, niacin coaxes the liver into increasing HDL levels, and lowering LDL and triglyceride levels. The dose response for niacin is linear. However, high doses of niacin are associated with hepatotoxicity and hyperhomocysteinemia which is a factor in the development of atherosclerotic disease (Basu and Mann, Vitamin B-6 normalizes the altered sulfur amino acid status of rats fed diets containing pharmacological levels of niacin without reducing niacin's hypolipidemic effects, J Nutr. 1997 Jan;127(1):117-21). The use of niacin is also associated with an increase in thromboxane A_2, which is related to certain cardiovascular diseases including myocardial infarction, angina, and cerebral ischemia and is also involved in platelet/vessel wall interaction.

The inventors have discovered that niacin and P5P or certain P5P related compounds in combination reduce the risk of cardiovascular disease and diabetes in a synergistic manner with substantially no incidence of hepatotoxicity. The inventors have discovered that the lipid lowering properties of niacin and P5P or P5P related compounds are synergized when coadministered. The inventors have also discovered that P5P and P5P related compounds are capable of ameliorating niacin

mediated increases in homocysteine and thromboxane A2 levels, without altering the hypolipdiemic action of niacin.

Fibrates, which are also known as fibric acid derivatives, have also been used to lower the risk of heart disease. Fibric acid derivatives increase lipoprotein lipase activity in adipose tissue, thereby increasing the catabolism of VLDL. Fibric acid derivatives also reduce triglyceride levels, modestly reduce LDL levels and raise HDL levels. Fibric acid derivatives are associated with an increased risk of gastrointestinal and hepatobilary neoplasia. Fibric acid derivatives are also known to substantially increase homocysteine levels thereby counteracting the cardioprotective protective effect of the drug. The use of fibric acid derivatives in combination with vitamin B6 (pyroxidine) has been previously reported by Dierkes et al, 2001 (Vitamin supplementation can markedly reduce the homocysteine elevation induced by fenofibrate, Atherosclerosis. 2001 Sep;158(1):161-4). Dierkes et al showed that treating patients with fenofibrate and a placebo increased homocysteine levels by 44% whereas patients given fenofibrate, vitamin B6, B12, and folic acid only increased homocysteine levels by 13%.

The inventors have now discovered that P5P and P5P related compounds are more effective than vitamin B6 at reducing fibrate induced hyperhomocysteinemia. The inventors have also discovered that the lipid lowering properties of fibric acid derivatives and P5P and P5P related compounds are synergized with substantially no incidence of hepatotoxicity.

PLA_2 has been indicated as is a strong independent risk factor for coronary heart disease (Camejo et al, Phospholipase A_2 in Vascular Disease, Circ Res. 2001, 89:298:304 at 298) and is also considered an inflammatory biomarker. PLA_2 catalyses the hydrolysis of the sn-2 ester bond in glyceroacyl phospholipids present in lipoproteins and cell membranes forming non-esterified fatty acids and lysophopholipids.

PLA_2 plays a role in several processes which increase the risk for cardiovascular disease. PLA_2 can modify circulating lipoproteins and induce the formation of LDL particles associated with increased risk for cardiovascular disease (Camejo et al., 2001, at p. 298). In arterial walls, PLA_2 can induce aggregation and fusion of matrix-bound lipoproteins and further increase their binding strength to matrix proteoglycans. PLA_2 catalyzes the release of arachidonic acid from cell membranes which is converted by cycloxygenases to thromboxanes which promote vasoconstriction and platelet adhesion. Arachidonic acid is also converted by cycloxygenases to prostaglandins which mediate inflammation, a further cardiovascular disease risk factor. Prostaglandins and other inflammatory mediators influence multiple processes, including cholesterol homeostasis and coagulation.

P5P, a vitamin B6 metabolite, has been implicated in the inhibition of arachidonic acid release via PLA_2 activation (Krinshnamurthi and Kakkar, Effect of pyridoxal 5'phosphate (PALP) on human platelet aggregation, dense granule release and thromboxane B2 generation – role of Schiff base formation, Thromb Haemost. 1982, 48:136). Thus, P5P and P5P related compounds provide cardioprotective benefits by regulating PLA_2 levels in addition to lipoprotein levels.

The present inventors are the first to employ a pyridoxal-5'-phosphate or pyridoxal-5'-phosphate related compound as an active agent for the reduction of cholesterol and PLA_2 in combination with a nicotinic acid derivative or a fibric acid derivative. The present inventors have discovered that the lipid lowering and PLA_2 inhibition properties of P5P and P5P related compounds are significantly greater than those for vitamin B6 and other previously disclosed vitamin B6 derivatives (see US Patent 6,066,659 and German patent DE 24 61 742 C2). P5P is forty times more potent in vivo as compared to pyroxidine. The inventors have also discovered that cardiovascular protective effects of P5P and P5P related compounds in combination with a nicotinic acid derivative or a fibric acid derivative are synergized when they are administered in combination. The inventors have further discovered that P5P and P5P related compounds and a nicotinic acid derivative or a fibric acid derivative

do not react adversely when coadministered. P5P and P5P related compounds do not inhibit hepatic CYP enzymes and do not increase hepatic transaminases. Accordingly, the pharmaceutical compounds of the present invention are non-hepatotoxic.

In light of these discoveries, the present invention provides pharmaceutical compositions and uses thereof for treating or preventing hypercholesterolemia, reducing the risk of cardiovascular disease and diabetes. The pharmaceutical compositions of the present invention are more effective than currently available combination therapies in reducing risk of cardiovascular disease. The pharmaceutical compositions ameliorate multiple risk factors including lipoproteins, homocysteine, vasoconstriction, platelet aggregation and inflammation. Furthermore, the pharmaceutical compositions do not induce hepatotoxicity. The pharmaceutical compositions of the present invention are comprised of: a nicotinic acid derivative or a fibric acid derivative; a pyridoxal-5'-phosphate or pyridoxal-5'-phosphate related compound or a pharmaceutically acceptable salt thereof; and a pharmaceutically acceptable carrier.

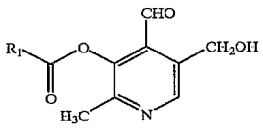
Examples of fibric acid derivatives that may be used include but are not limited to bezafibrate, clofibrate, ciprofibrate, fenofibrate (Tricor ^{TM}), or gemifibrozil (Lopoid ^{TM}). Preferably, the fibric acid derivative is fenofibrate.

Examples of nicotinic acid derivatives that may be used include niacin, niceritrol, acipimox, and acifran. Preferably the nicotinic acid derivative is niacin.

Examples of the pyridoxal-5'-phosphate related compound which may be used include but are not limited to pyridoxal-5-phosphate (P5P), pyridoxal, and pyridoxamine. Other P5P related compounds, which can also be used, include the 3-acylated analogues of pyridoxal, 3'acylated analogues of pyridoxal-4,5-aminal, and pyridoxine phosphonate analogues as disclosed in US Patent No. 6,585,414 and U.S. Patent Application No. 20030114424, both of which are incorporated herein by

reference. Preferably, the pyridoxal-5'-phosphate or pyridoxal-5'-phosphate related compound will be P5P.

The 3-acylated analogues of pyridoxal include:



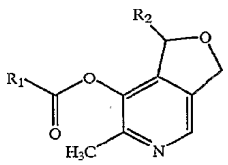
wherein,

R_1 is alkyl, alkenyl, in which alkyl can interrupted by nitrogen, oxygen, or sulfur, and can be unsubstituted or substituted at the terminal carbon with hydroxy, alkoxy, alkanoyloxy, alkoxyalkanoyl, alkoxycarbonyl, or

R_1 is dialkylcarbamoyloxy; alkoxy; dialkylamino; alkanoyloxy; alkanoyloxyaryl; alkoxyalkanoyl; alkoxycarbonyl; dialkylcarbamoyloxy; or

R_1 is aryl, aryloxy, arylthio, or aralkyl, in which aryl can be substituted by alkyl, alkoxy, amino, hydroxy, halo, nitro, or alkanoyloxy.

The 3-acylated analogues of pyridoxal-4,5-aminal include:



wherein,

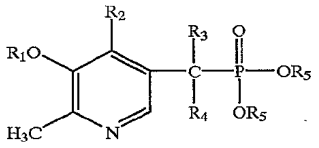
R_1 is alkyl, alkenyl, in which alkyl can interrupted by nitrogen, oxygen, or sulfur, and can be unsubstituted or substituted at the terminal carbon with hydroxy, alkoxy, alkanoyloxy, alkoxyalkanoyl, alkoxycarbonyl, or

R_1 is dialkylcarbamoyloxy; alkoxy; dialkylamino; alkanoyloxy; alkanoyloxyaryl; alkoxyalkanoyl; alkoxycarbonyl; dialkylcarbamoyloxy; or

R_1 is aryl, aryloxy, arylthio, or aralkyl, in which aryl can be substituted by alkyl, alkoxy, amino, hydroxy, halo, nitro, or alkanoyloxy;

R_2 is a secondary amino group.

The pyridoxine phosphate analogues include:

(a) 

wherein,

R_1 is hydrogen or alkyl;

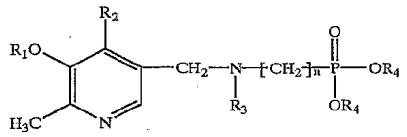
R_2 is –CHO-, -CH_2OH, -CH_3, -CO_2R6 in which R6 is hydrogen, alkyl, aryl; or

R_2 is –CH_2-O alkyl in which alkyl is covalently bonded to the oxygen at the 3-position instead of R_1;

R_3 is hydrogen and R_4 is hydroxy, halo, alkoxy, alkanoyloxy, alkylamino, or arylamino; or

R_3 and R_4 are halo; and

R_5 is hydrogen, alkyl, aryl, aralkyl, or $-CO_2R_7$ in which R_7 is hydrogen, alkyl, aryl, or aralkyl;

(b) 

wherein,

R_1 is hydrogen or alkyl;

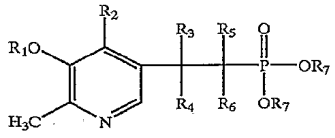
R_2 is –CHO, $-CH_2OH$, $-CH_3$, $-CO_2R_5$ in which R_5 is hydrogen, alkyl, aryl; or

R_2 is $-CH_2$-O alkyl in which alkyl is covalently bonded to the oxygen at the 3-position instead of R_1;

R_3 is hydrogen, alkyl, aryl, aralkyl,

R_4 is hydrogen, alkyl, aryl, aralkyl, or $-CO_2R6$ in which R6 is hydrogen, alkyl, aryl or aralkyl;

n is 1 to 6; and

(c) 

wherein,

R_1 is hydrogen or alkyl;

R_2 is –CHO-, CH_2OH-, -CH_3, -CO_2R_8 in which R_8 is hydrogen, alkyl, aryl; or

R_2 is –CH_2-O alkyl- in which alkyl is covalently bonded to the oxygen at the 3-position instead of R_1;

R_3 is hydrogen and R_4 is hydroxy, halo, alkoxy, or alkanoyloxy; or

R_3 and R_4 can be taken together to form =O;

R_5 and R6 are hydrogen; or

R_5 and R6 are halo;

R_7 is hydrogen, alkyl, aryl, aralkyl, or –CO_2R_8 in which R_8 is hydrogen, alkyl, aryl, or aralkyl.

It is to be understood that this invention is not limited to specific dosage forms, carriers, or the like, as such may vary. It is also to be understood that the terminology used herein is for the purpose of describing particular embodiments only, and is not intended to be limiting.

Some of the compounds described herein contain one or more asymmetric centres and this may give raise to enantiomers, disasteriomers, and other stereroisomeric forms which may be defined in terms of absolute stereochemistry as (R)- or (S)-. The present invention is meant to include all such possible diasteriomers and enantiomers as well as their racemic and optically pure forms. Optically active (R)- and (S)- isomers may be prepared using chiral synthons or chiral reagents, or resolved using conventional techniques. When the compounds described herein contain olefinic double bonds or other centres of geometric symmetry, and unless specified otherwise, it is intended that the compounds include both E and A geometric isomers. Likewise all tautomeric forms are intended to be included.

As used in this specification and the appended claims, the singular forms "a," "an", and "the" include plural referents unless the context clearly dictates otherwise. Thus, for example, reference to "an active agent" or "a pharmacologically active agent" includes a single active agent as well as two or more different active agents in combination, reference to "a carrier" includes mixtures of two or more carriers as well as a single carrier, and the like.

By "pharmaceutically acceptable," such as in the recitation of a "pharmaceutically acceptable carrier," or a "pharmaceutically acceptable salt," is meant herein a material that is not biologically or otherwise undesirable, i.e., the material may be incorporated into a pharmaceutical composition administered to a patient without causing any undesirable biological effects or interacting in a deleterious manner with any of the other components of the composition in which it is contained.

"Carriers" or "vehicles" as used herein refer to conventional pharmaceutically acceptable carrier materials suitable for drug administration, and include any such materials known in the art that are nontoxic and do not interact with other components of a pharmaceutical composition or drug delivery system in a deleterious manner.

By an "effective" amount or a "therapeutically effective amount" of a drug or pharmacologically active agent is meant a nontoxic but sufficient amount of the drug or agent to provide the desired effect. In the combination therapy of the present invention, an "effective amount" of one component of the combination is the amount of that compound that is effective to provide the desired effect when used in combination with the other components of the combination. The amount that is "effective" will vary from subject to subject, depending on the age and general condition of the individual, the particular active agent or agents, and the like. Thus, it is not always possible to specify an exact "effective amount." However, an appropriate "effective" amount in any individual case may be determined by one of ordinary skill in the art using routine experimentation.

The terms "reduce the risk of cardiovascular disease" and "reducing the risk of cardiovascular disease" as used herein refer to the reduction or elimination of an underlying cause or biomarker associated with the increased incidence of a cardiovascular event.

As used herein, "cardiovascular disease" means any disease of the heart of blood vessels. Examples of cardiovascular disease include: congestive heart failure, myocardial ischemia, arrhythmia, myocardial infarction, ischemic stroke, hemorrhagic stroke, coronary artery disease, hypertension (high blood pressure), atherosclerosis (clogging of the arteries), aneurysm, peripheral artery disease (PAD), thrombophlebitis (vein inflammation), diseases of the heart lining, diseases of the heart muscle, carditis, congestive heart failure, endocarditis, ischemic heart disease, valvular heart disease (malfunction of a valve or valves in the blood vessels of the heart), arteriosclerosis (hardening of the arteries), acute coronary syndrome (ACS), high cholesterol, deep vein thrombosis (DVT), Kawazaki disease, and heart transplant.

The terms "reduce the risk of diabetes" and "reducing the risk of diabetes" as used herein refer to the reduction or elimination of an underlying cause or biomarker associated with the increased incidence of developing insulin resistance, pre-diabetes and diabetes.

As used herein, "pyridoxal-5'-phosphate or pyridoxal-5'-phosphate related compound", means any vitamin B6 precursor, metabolite, derivative, or analogue thereof but excludes: (1) vitamin B6 (pyroxidine); (2) the 5' phosphoric acid esters of pyridoxal, pyridoxol and pyridoxamine disclosed in German Patent DE 24 61 742 C2, and (3) the pyridoxine, pyridoxal, and pyridoxamine derivatives disclosed in US Patent No. 6,066,659).

As used herein, "hepatotoxicity" includes any drug-induced liver injury.

The pharmaceutical compositions of the present invention may be manufactured in a manner that is itself known, e.g., by means of conventional mixing, dissolving, granulating, dragee-making, levigating, emulsifying, encapsulating, entrapping or lyophilizing processes.

Pharmaceutical compositions for use in accordance with the present invention thus may be formulated in conventional manner using one or more physiologically acceptable carriers comprising excipients and auxiliaries which facilitate processing of the active compounds into preparations which can be used pharmaceutically. Proper formulation is dependent upon the route of administration chosen.

For injection, the agents of the invention may be formulated in aqueous solutions, preferably in physiologically compatible buffers such as Hanks' solution, Ringer's solution, or physiological saline buffer.

For oral administration, the compounds can be formulated readily by combining the active compounds with pharmaceutically acceptable carriers well known in the art. Such carriers enable the compounds of the invention to be formulated as tablets, pills, dragees, capsules, liquids, gels, syrups, slurries, suspensions and the like, for oral ingestion by a patient to be treated. Pharmaceutical preparations for oral use can be obtained by solid excipient, optionally grinding a resulting mixture, and processing the mixture of granules, after adding suitable auxiliaries, if desired, to obtain tablets or dragee cores. Suitable excipients are, in particular, fillers such as sugars, including lactose, sucrose, mannitol, or sorbitol, or cellulose preparations such as, maize starch, wheat starch, rice starch, potato starch, gelatin, gum tragacanth, methyl cellulose, hydroxypropylmethyl-cellulose, sodium carboxymethylcellulose, and/or polyvinylpyrrolidone. If desired, disintegrating agents may be added, such as the cross-linked polyvinylpyrrolidone, agar, or alginic acid or a salt thereof such as sodium alginate.

Preferably, the pharmaceutical compositions of the present invention are administered orally. Preferred oral dosage forms contain a therapeutically effective unit dose of each active agent, wherein the unit dose is suitable for a once-daily oral administration. The therapeutic effective unit does of any of the active agents will depend on number of factors which will be apparent to those skilled in the art and in light of the disclosure herein. In particular these factors include: the identity of the compounds to be administered, the formulation, the route of administration employed, the patient's gender, age, and weight, and the severity of the condition being treated and the presence of concurrent illness affecting the gastro-intestinal tract, the hepatobillary system and the renal system. Methods for determining dosage and toxicity are well known in the art with studies generally beginning in animals and then in humans if no significant animal toxicity is observed. The appropriateness of the dosage can be assessed by monitoring LDL levels, HDL levels, total cholesterol levels, triglycerides levels, and homocysteine levels. Where the dose provided does not cause LDL lipoprotein and homocysteine levels to decline to normal or tolerable levels, following at least 2 to 4 weeks of treatment, the dose can be increased.

The therapeutic effective unit dosage for a nicotinic acid derivative is between 100 mg and 5000 mg per day. Preferably, the unit dosage is between 250 mg and 3000 mg per day. Typically the unit dosage will be 100, 250, 500, 1000, or 3000 mg per day.

The therapeutic effective unit dosage for the fibric acid derivative is between 100 mg and 1000 mg per day. Suitable dosage ranges for particular fibric acid derivatives are known in the art. Typically the unit dosage will be 100, 200, 400, or 600 mg per day. Where the fibric acid derivative employed is bezafibrate, the preferred unit dosage is 400 mg/day. Where the fibric acid derivative employed is ciprofibrate, the preferred unit dosage is 200 mg/day. Where the fibric acid derivative employed is gemfibrozil, the preferred unit dosage is 600 mg/day. Where the fibric acid derivative employed is fenofibrate, the preferred unit dosage is 200 mg/day.

The preferred therapeutic effective unit dosage for the pyridoxal-5'-phosphate or pyridoxal-5'-phosphate related compound is between 0.1 to 50 mg/kg body weight daily. More preferably, the unit dosage will be 1 to 15 mg/kg body weight daily. In embodiments, the dose is 10mg/kg/day, alternatively 250 mg/day, 500 mg/day or 750 mg/day.

Although the present invention has been described with reference to illustrative embodiments, it is to be understood that the invention is not limited to these precise embodiments, and that various changes and modifications may be effected therein by one skilled in the art. All such changes and modifications are intended to be encompassed by the appended claims.

Example One – Effect of P5P on CYP Activity

The inhibitory effect of P5P on the activity of hepatic cytochrome enzymes was examined *in vitro*. The CYP inhibition assays used microsomes (Supersomes®, Gentest Corp., Woburn, MA) prepared from insect cells, each expressing an individual CYP subtype (CYP1A2, CYP2A6, CYP2B6, CYP2C8, CYP2C9, CYP2C19, CYP2D6, CYP2E1 or CYP3A4) expressed from the corresponding human CYP cDNA using a baculovirus expression vector. The microsomes also incorporated supplemental cDNA-expressed human reductase and/or cytochrome b5, as these enzymes stimulate the activity of the CYPs, allowing for a reduction in the amount of enzyme required per reaction (Gentest Corp.). The assays monitored, *via* fluorescence detection, the formation of a fluorescent metabolite following incubation of the microsomes with a specific CYP substrate. Two CYP substrates (7-benzyloxy-4-trifluoromethylcoumarin (BFC) and 7-benzyloxycoumarin (BQ)) were tested for CYP3A4, as this enzyme has been shown to exhibit complex inhibition kinetics. Reactions (0.2 mL) were performed in 96-well microtitre plates at 37°C in the presence of an NADPH regenerating system [NADP+, glucose-6-phosphate

(G6P), glucose-6-phosphate dehydrogenase (G6PDH)] and $MgCl_2$. Inhibition of metabolic product formation by pyridoxal 5'-phosphate for each enzyme was tested in the absence (0 µM) and presence of 0.0169 to 37.0 µM pyridoxal 5'-phosphate. An enzyme-selective inhibitor was also tested at 8 concentrations in each assay as a positive control. All determinations were performed in duplicate. The reagent solutions used for all of the CYP subtype assays, except CYP2C19 and CYP3A4, were prepared by MBDI. For CYP2C19 and CYP3A4, complete reagent kits purchased from Gentest Corp. (CYP2C19/CEC: Cat. No. HTS-4000, Lot No. 1; CYP3A4/BFC: Cat. No. HTS-1000, Lot No. 1) were used to perform the assays.

Experiment Design - Assays for all enzymes were performed in the following manner: the NADPH regenerating system, appropriate buffer solution and vehicle, inhibitor (positive control) solution or test compound (pyridoxal 5'-phosphate) solution were dispensed into 96-well microtitre plates. Eight inhibitor and test compound concentrations were tested using 3-fold serial dilutions. The microtitre plates containing 0.1 mL/well of the latter mixture were pre-warmed to 37°C in an incubator. A solution of buffer, microsomes and substrate was separately prepared and vortex mixed to disperse the protein. The reactions were initiated by the addition of the microsome/substrate solution (0.1 mL) to the wells of the microtitre plates containing the pre-warmed NADPH regenerating system, buffer and inhibitor solutions. Following specified incubation times, the reactions were stopped by the addition of 0.075 mL of a STOP solution (see below). Blank (background noise) samples were also assayed by adding the STOP solution prior to the addition of the microsome/substrate mix to the NADPH regenerating system. The amount of metabolic product formed was quantified by fluorescence detection in a fluorescence plate reader utilizing excitation and emission filters that had been optimized for the detection of each metabolite.

Prior to performing the CYP inhibition assays, the effect of pyridoxal 5'-phosphate on the fluorescence of the metabolic products measured in the assays was evaluated.

The fluorescence of metabolite (one concentration, in duplicate) was measured in the absence (0 µM) and presence of 0.457 to 1000 µM pyridoxal 5'-phosphate. The concentrations and metabolic products measured were: 1 µM 3-cyano-7-hydroxycoumarin (CHC), 2.5 µM 7-hydroxycoumarin (7-HC), 2.5 µM 7-hydroxy-4-trifluoromethylcoumarin (HFC), 0.1 µM fluorescein, 10 µM 3-[2-(N,N-diethylamino)ethyl]-7-hydroxy-4-methylcoumarin (AHMC) and 10 µM quinolinol. The concentration of metabolite used was based on the expected maximum concentration of metabolite formed in the CYP inhibition assay (*i.e.* the concentration of metabolite measured following incubation substrate with the CYP subtype in the absence of an inhibitor). CHC was the fluorescent metabolite measured in the CYP1A2 and CYP2C19 assays. 7-HC was the fluorescent metabolite measured in the CYP2A6 assay, HFC was the fluorescent metabolite measured in the CYP2B6, CYP2C9, CYP2E1 and CYP3A4 (BFC as substrate) assays and fluorescein was the metabolite measured in the CYP2C8 assay. AHMC was the metabolite measured in the CYP2D6 assay and quinolinol was measured in the CYP3A4 (BQ as substrate) assay.

Pyridoxal 5'-Phosphate Solution - pyridoxal 5'-phosphate monohydrate (P5P, Lot No. 00001448) was supplied as powder. The concentrations of all pyridoxal 5'-phosphate solutions are based on the anhydrous molecular weight (247.15 g/mole) corrected for a potency factor of 0.9019.

For the determination of the effect of pyridoxal 5'-phosphate on metabolite fluorescence, a stock solution of pyridoxal 5'-phosphate, at a concentration of 50 mM, was freshly prepared in distilled water. Since pyridoxal 5'-phosphate is acidic in aqueous solution, the pH of the solution was adjusted to 7.0 with 1 N NaOH. The solution of pyridoxal 5'-phosphate was added to the wells of the microtitre plate starting with a 50-fold dilution to 1000 µM, followed by 3-fold serial dilutions to: 333, 111, 37.0, 12.3, 4.12, 1.37 and 0.457 µM.

For the CYP subtype inhibition assays, a stock solution of pyridoxal 5'-phosphate, at a concentration of 50 mM, was freshly prepared in distilled water (pH adjusted to 7.0 with 1 N NaOH). The solution of pyridoxal 5'-phosphate was diluted with distilled water to 111 µM and then added to the wells of the microtitre plate starting with a 3-fold dilution to 37.0 µM, followed by 3-fold serial dilutions to: 12.4, 4.12, 1.37, 0.457, 0.152, 0.0508 and 0.0169 µM.

Data Analysis - The mean of the duplicate fluorescent signals in the presence and absence (vehicle control) of each compound was calculated and corrected for the background noise. Percent inhibition was calculated as the difference in the corrected fluorescent signals in the absence and presence of the compound, divided by the corrected fluorescent signal in the absence of compound, multiplied by 100%. The concentration of the inhibitor or pyridoxal 5'-phosphate, where appropriate, which inhibited metabolite formation by 50% (IC_{50}) was calculated by nonlinear regression analysis (sigmoidal dose-response curve) of the % Inhibition *versus* Log concentration data using GraphPad Prism software (Version 3.00, GraphPad Software, Inc., San Diego, CA).

Results - The effect of pyridoxal 5'-phosphate on the fluorescence of the various metabolic products measured in the CYP inhibition assays was determined. As evident in Figure 1, pyridoxal 5'-phosphate significantly quenched (decreased) the fluorescence of five the six metabolites measured in the assays (CHC, 7-HC, 7-HFC, AHMC and quinolinol) at concentrations of > 37 µM. Figures 1(a) to 1(f), illustrate the decrease in the fluorescence of the metabolic products (CHC, 7-HC, HFC, fluorescein, AHMC and quinolinol) measured in the CYP inhibition assays as a function of pyridoxal 5'-phosphate concentration. Pyridoxal 5'-phosphate (up to 1000 µM) did not affect the fluorescence of fluorescein, the metabolic product measured following the metabolism of dibenzylfluorescein by the CYP2C8 enzyme. The inhibitory effect of pyridoxal 5'-phosphate on CYP catalytic activity was tested over the concentration range of 0.0169 to 37 µM.

The results from the incubation of the known inhibitors and pyridoxal 5'-phosphate with each of the CYP subtypes (CYP1A2, CYP2A6, CYP2B6, CYP2C8, CYP2C9, CYP2C19, CYP2D6, CYP2E1 and CYP3A4) are depicted graphically in Figures 2 to 11.

Figures 2(a) and 2(b) illustrate the inhibition of the catalytic activity of CYP1A2 (metabolism of CEC to CHC) as a function of Furafylline and P5P concentration respectively.

Figures 3(a) and 3(b) illustrate the inhibition of the catalytic activity of CYP2A6 (metabolism of coumarin to 7-HC) as a function of Tranylcypromine and P5P concentration respectively.

Figures 4(a) and 4(b) illustrate the inhibition of the catalytic activity of CYP2B6 (metabolism of EFC to HFC) as a function of Tranylcypromine and P5P concentration respectively.

Figures 5(a) and 5(b) illustrate the inhibition of the catalytic activity of CYP2C8 (metabolism of DBF to Fluorescein) as a function of Quercetin and P5P concentration respectively.

Figures 6(a) and 6(b) illustrate the inhibition of the catalytic activity of CYP2C9 (metabolism of MFC to HFC) as a function of Sulfaphenazole and P5P concentration respectively.

Figures 7(a) and 7(b) illustrate the inhibition of catalytic activity of CYP2C19 (metabolism of CEC to CHC) as a function of Tranylcypromine and P5P concentration respectively.

Figures 8(a) and 8(b) illustrate the inhibition of the catalytic activity of CYP2D6 (metabolism of AMMC to AHMC) as a function of Quinidine and P5P concentration respectively.

Figures graphs 9(a) and 9(b) illustrate the inhibition of the catalytic activity of CYP2E1 (metabolism of MFC to HFC) as a function of Diethyldithiocarbamic acid (DDTC) and P5P concentration respectively.

Figures 10(a) and 10(b) illustrate the inhibition of the catalytic activity of CYP3A4 (metabolism of BFC to HFC) as a function of Ketoconazole and P5P concentration respectively.

Figures 11(a) and 11(b) illustrate the inhibition of the catalytic activity of CYP3A4 (metabolism of BQ to Quinolinol) as a function of Ketoconazole and P5P concentration.

Figure 12 summarizes the IC_{50} values estimated for the known inhibitors of each CYP subtype, and for pyridoxal 5'-phosphate.

The observed IC_{50} values for the various CYP inhibitors are similar to those obtained previously in our laboratory during assay validation (for CYP1A2, CYP2A6, CYP2B6, CYP2C8, CYP2C9, CYP2D6 and CYP2E1) and are similar to those determined by the supplier (for the CYP2C19 and CYP3A4 assay kits). These data indicate that enzyme activity was not compromised in any of the assays.

Over the concentration range tested (0.0169 to 37.0 µM), pyridoxal 5'-phosphate did not inhibit the catalytic acitivity of seven of the CYP enzymes: CYP1A2, CYP2A6, CYP2B6, CYP2C8, CYP2C9, CYP2D6 and CYP2E1 (Figures 2, 3, 4, 5, 6, 8 and 9, respectively). Pyridoxal 5'-phosphate did, however, inhibit the metabolic activity of the CYP2C19 and CYP3A4 enzyme subtypes (Figures 7, 10 and 11). The potency of pyridoxal 5'-phosphate was relatively similar for the CYP2C19 and CYP3A4

enzyme subtypes (IC_{50} values of ~33 and ~37 µM, respectively). Pyridoxal 5'-phosphate appeared to inhibit the CYP3A4 enzyme-mediated metabolism of the substrate BFC to a slightly greater extent ($IC_{50} \cong 37$ µM) than the substrate BQ (IC_{50} >37 µM, Figures 10 and 11, respectively). A summary of the IC_{50} values for pyridoxal 5'-phosphate and the known inhibitors is given in Figure 12.

Conclusions - The compound pyridoxal 5'-phosphate did not selectively inhibit the catalytic activity of seven CYP subtypes: CYP1A2, CYP2A6, CYP2B6, CYP2C8, CYP2C9, CYP2D6 and CYP2E1, over the concentration range tested (0.0169 to 37.0 µM). Clinically relevant drug interactions would, therefore, not be expected to occur between pyridoxal 5'-phosphate and substrates of these enzymes. Pyridoxal 5'-phosphate did selectively inhibit the catalytic activity of two (CYP2C19 and CYP3A4) of the nine human CYP subtypes tested at relatively high concentrations (IC_{50} = 33 µM for CYP2C19 and ≥37 µM for CYP3A4). However, based on the relatively low inhibitory potency of pyridoxal 5'-phosphate for the two CYP subtypes *in vitro*, the occurrence of serious drug interactions is expected to be unlikely.

Example Two - Effectiveness of pyridoxal-5'-phosphate for the reduction of myocardial ischemic injury following coronary intervention

Methods - Study Overview: 60 patients who underwent percutaneous coronary intervention (PCI) at 4 centers were randomized in a 2:1 double-blinded fashion to treatment with P5P or placebo. Inclusion criteria required prior determination for non-urgent PCI of a single-vessel lesion(s) and identification of = 1 of the following clinical characteristics determining high risk for procedural-related ischemic complications (Califf RM, Abdelmeguid AE, Kuntz RE, Popma JJ, Davidson CJ, Cohen EA, Kleiman NS, Mahaffey KW, Topol EJ, Pepine CJ, et al. Myonecrosis after revascularization procedures. J Am Coll Cardiol 1998; 31:241–251; The ESPRIT Investigators. Novel dosing regimen of eptifibatide in planned coronary stent implantation: a randomised, placebo-controlled trial. Lancet 2000; 356:2037–2044): presence of an acute coronary syndrome (chest pain within 48 hours of PCI), recent

AMI (= 7 days), diminished epicardial blood flow, angiographic thrombus, ejection fraction = 30%, or vein graft lesion. In addition to any general contraindication to the PCI procedure or standard concomitant therapies, major exclusion criteria were creatine kinase (CK-MB) elevation above the upper limit of normal immediately before PCI, electrocardiographic evidence of atrial fibrillation or left bundle branch block, or evidence of any clinically significant abnormal laboratory finding (transaminases, bilirubin, or alkaline phosphatase >1.5 times the upper limit of normal or serum creatinine >1.8 mg/dl). Patients with elevated troponin measurements were permitted in the study provided that the peak troponin value was reported >24 hours before scheduled PCI, with documentation of a decreasing value before revascularization. After providing informed consent, patients randomized to treatment with P5P were administered enteric-coated P5P as a 10 mg/kg oral dose = 4 hours before PCI followed by 2 daily doses of 5 mg/kg orally for 14 days. Compliance and reasons for discontinued treatments were recorded for all patients.

Study end points and definitions - The primary objective of the study was to evaluate the feasibility of treatment with P5P as a cardioprotective agent in high-risk elective PCI. The primary end point of infarct size was evaluated by the trapezoidal rule (Press WH, Teukolsky SA, Vetterling WT, Flannery BP. Numerical Recipes. Cambridge, UK: Cambridge University Press, 1994:127–133.) using serial CK-MB enzyme measures performed at baseline and every 6 hours for 24 hours beginning immediately before initiation of PCI. The occurrence of myocardial ischemia within 24 hours after PCI was assessed as a secondary end point using continuous 12-lead electrocardiographic monitoring (Northeast Monitoring, Boston, Massachusetts). Evidence of periprocedural ischemia was defined as ST-segment depression of = 100 μV within a 60-minute period of PCI, lasting = 1 minute and separated from other episodes by = 1 minute. Area under the curve ST-segment deviation was measured from the onset of the first to the last contrast injection. All cardiac markers and ST-segment monitoring data were analyzed by core laboratories blinded to treatment

assignment (University of Maryland School of Medicine, Baltimore, Maryland; Duke Ischemia Monitoring Laboratory, Durham, North Carolina). Additional prespecified secondary end points included the 30-day composite and individual event rates of death; nonfatal infarction; new or worsening heart failure, or recurrent ischemia in addition to net clinical safety, which was defined as the absence of major adverse ischemic events; Thrombolysis In Myocardial Infarction (TIMI) major bleeding; and liver function or coagulation test abnormalities. Acute myocardial infarction (AMI) was defined as CK-MB elevation = 3 times the upper limit of normal (upper limit of normal 7 ng/ml) and/or troponin T levels = 1.5 times the upper limit of normal (upper limit of normal 0.1 ng/ml). If previous troponin (or CKMB) values were above the upper limit of normal, values were required to be >50% of the baseline measurement in addition to = 2 times (= 3 times for CK-MB) the upper limit of normal to meet the definition of AMI. Routine chemistries, complete blood count, and coagulation assays were performed at baseline, 7 days, and 30 days after randomization. Peak periprocedural CK-MB and the maximum difference in troponin levels from baseline to within 24 hours after PCI were also examined.

Data collection and statistical analyses - Patients who received = 1 dose of the study drug and underwent PCI were analyzed for all primary and secondary efficacy and safety end points. Patients who received = 1 dose of study drug but who did not undergo PCI were excluded from the primary efficacy and ST segment monitoring analyses but were included in the safety analyses. Statistical tests were 2-sided with an a level of 0.05 and employed the intent-to-treat principle. The Wilcoxon rank-sum test was used. Due to small sample sizes, categorical variables were compared using the Fisher's exact test with the exception of the ST-segment monitoring data, which utilized the Pearson's chi-square test. Statistical analyses were performed using SAS version 8.2 (SAS Institute, Cary, North Carolina).

Results - Of the 60 patients enrolled in the study of P5P in high-risk PCI, all patients received treatment with P5P or placebo; however, 4 patients (3 P5P, 1 placebo) did not undergo planned revascularization. An additional 3 patients were excluded from

the area under the curve analyses due to incomplete collection of cardiac enzyme data. As a result, 53 and 60 patients were included in the primary efficacy and 30-day clinical and/or safety analyses, respectively.

The presence of established cardiovascular disease, prior revascularization, and cardiovascular risk factors were similar between patients randomized to P5P or placebo and representative of patient populations in larger contemporary trials that studied patients with acute coronary syndromes. Table 1 summarizes baseline clinical, electrocardiographic, and angiographic characteristics in patients treated with P5P or placebo. Overall, the mean age of the population was 58 years, 81.7% of patients were men, and 21.7% had undergone previous PCI and/or bypass surgery. Although recent AMI as an indication for revascularization occurred more commonly among patients treated with P5P, a similar number of patients in each group presented with an acute coronary syndrome, and approximately half of all patients had elevated troponin levels before PCI.

Except for a higher incidence of reduced epicardial flow among control patients, baseline angiographic and procedural characteristics also appeared similar between treatment groups (Table 1). Administration of P5P or placebo occurred an average of 6.1 and 8.4 hours before PCI, respectively. Stent implantation was performed in 100% and 97.3% of the placebo and P5P treatment groups, respectively. Only 1 vein graft intervention was performed using distal embolic protection. Although the right coronary artery was most commonly treated in both groups, fewer patients treated with placebo underwent revascularization of a saphenous vein graft. Table 2 summarizes procedural and angiographic results for patients treated with P5P or placebo. Procedural angiographic complications (e.g., major dissection, abrupt vessel closure) were infrequent (Table 2).

The primary end point of periprocedural infarct size measured according to median periprocedural CK-MB area under the curve was reduced from 32.9 to 18.6 ng/ml ($p= 0.038$), reflecting a shift in the distribution of CK-MB. Table 3 summarizes

periprocedural cardiac markers and ST monitoring results for patients treated with P5P or placebo. Figure 13 illustrates the area under the curve CK-MB values fitted to a log-normal distribution for patients treated with P5P (A) and placebo (B). Similarly, the maximum periprocedural CK-MB level was significantly lower among patients receiving P5P. By categorical classification, the occurrence of 30-day nonfatal AMI did not differ between groups (12.8% with P5P vs 10.0% with placebo, p = 1.0). There were no deaths, and 30-day composite adverse event rates (death, nonfatal AMI, new and/or worsening heart failure, or recurrent ischemia) were similar (17.9% with P5P vs 15.0% with placebo, p = 1.0).

Electrocardiographic ST monitoring data were available for 94.6% of the patients who underwent PCI and who received treatment (Table 3). Post-PCI ischemia occurred in approximately 15% of patients in both groups. Although lower rates of post-PCI ischemia were observed with P5P treatment (14.7% vs 17.6%, p = 0.78), there were no significant differences in ischemia parameters per continuous electrocardiographic monitoring (Table 3).

No safety issues related to treatment with P5P were identified. The occurrence of major bleeding (2.8% P5P vs 10.5% placebo, p = 0.27) and need for blood product transfusion (2.5% P5P vs 10.0% placebo, p = 0.26) was infrequent and did not significantly differ between groups. There were no apparent differences in abnormalities of routine chemistries or coagulation studies at 7 and 30 days. In both groups, however, approximately 1/4 of patients discontinued drug therapy before completion of the prescribed 2 weeks (30.8% P5P vs 25.0% placebo, p = 0.77). For patients taking P5P, but who did not undergo PCI (3 patients, 7.5%), the most common causes for early discontinuation were gastrointestinal intolerance followed by non-specific musculoskeletal pain.

Conclusion - In high-risk patients for periprocedural ischemic complications, treatment with the vitamin B6 metabolite P5P was associated with a decrease in myocardial injury, reflected by a reduction in the total amount of CK-MB released

after PCI. P5P therapy was associated with a significant decrease in peak periprocedural CK-MB elevation, a shift in the distribution of CK-MB to lower levels (Figure 13), and reduced periprocedural infarct size.

Example Three - Effectiveness of pyridoxal-5'-phosphate in combination with a Fibric acid derivatives for the reduction of myocardial ischemic injury following coronary intervention

Method - The study data of Example 1 was utilized. Of the 60 patients described in Example 1, patients who received adjunctive treatment with a fibric acid derivative (fenofibrate, 160 to 200 mg/day) in addition to P5P treatment were identified.

Results - In patients treated with P5P and fibric acid derivative, the secondary end point of maximum periprocedural CK-MB level was reduced from 3.41 ng/ml (placebo) to 0.75 ng/ml (P5P and fenofibrate),

Conclusions - P5P and fibric acid derivative combination therapy was associated with a significant decrease in peak periprocedural CK-MB elevation, and reduced periprocedural infarct size.

Example Four – Effect of P5P and Fibric Acid Derivative Combination Therapy on Atherosclerosis in Mice

Objectives - To investigate the anti-atherogenic effects of P5P in an animal model of atherosclerosis and to investigate the combination of P5P with a fibric acid derivative for enhanced or synergistic anti-atherogenic effect.

The study is designed to investigate the potential anti-atherogenic effects of P5P as compared with fenofibrate, and the combination of both in Apolipoprotein E-knockout (apoE-KO) mice. The study compares the effects of both drugs, alone and in

combination, on atherosclerotic lesion formation, plasma lipoproteins, lipoprotein oxidation, homocysteine levels, and markers for inflammation.

Drugs- The drug fenofibrate is available from Sigma. The dose of fenofibrate chosen for the present study is based on the doses used in previous studies with hyperlipidemic mice (Duez et al, Reduction of atherosclerosis by the peroxisome proliferator-activated receptor alpha agonist fenofibrate in mice. J Biol Chem. 2002 Dec 13;277(50):48051-7). The drug is mixed in the diet of mice at a concentration of 100 mg/kg body weight per day. P5P will be provided by CanAm Bioresearch Inc. The dose of 1 mg/kg body weight per day is mixed into the diet of the mice.

Experimental Design- Male apoE-/- mice on a C57BL/6J background (n=60) are purchased from Jackson Laboratories (Bar Harbor, Me). Mice are fed a chow diet and water ad libitum throughout the study. At 6 weeks of age, the animals are divided into four groups of 15 mice each, matched for their plasma lipid concentrations and body weight. The four groups of 15 mice are fed a PicoLab mouse diet (Jamieson's Pet Food Distributor) containing 9% (wt/wt) fat (controls), or the same diet supplemented with 100 mg/kg fenofibrate (fibrate group), 1 mg/kg P5P (P5P group), or 100 mg/kg fenofibrate + 1 mg/kg P5P (fibrate/P5P group). The animals are on the diets for 28 weeks, and are weighed biweekly. All animal experiments are approved by the institutional committee on animal welfare.

Lipid and Lipoprotein Analysis and Measurements of Fibrinogen, Serum Amyloid A, and von Willebrand Factor - After a 4-hour fasting period, blood samples are obtained by tail incision. Total plasma cholesterol, triglyceride levels, and lipoprotein profiles are measured at t = 0, 3, 6, 11, 19, 24, and 28 weeks as described elsewhere (Volger et al, Dietary plant stanol esters reduce VLDL cholesterol secretion and bile saturation in apolipoprotein e*3-leiden transgenic mice. Arterioscler Thromb Vasc Biol 2001; 21:1046–1052.). Plasma von Willebrand factor (vWF), serum amyloid A (SAA), and fibrinogen are measured by ELISA specific for vWF (Tranquille and Emeis, The simultaneous acute release of tissue-type

plasminogen activator and von Willebrand factor in the perfused rat hindleg region. Thromb Haemost 1990; 63:454–458.), SAA (Biosource), and fibrinogen (Kockx et al, Fibric acid derivatives suppress fibrinogen gene expression in rodents via activation of the peroxisome proliferator-activated receptor-[alpha]. Blood 1999; 93:2991–299).

Plasma Oxidized LDL Concentrations - Ninety-six–well polystyrene plates (Nunc) are coated with either oxidized LDL, at a concentration of 10 µg/mL in PBS, or native LDL (both from humans) overnight at 4°C. The subsequent steps are performed as described previously (10). IgG isotypes are determined with an ELISA kit (Southern Biotechnology).

Plasma Homocysteine Concentrations - Nonfasting blood samples are obtained and plasma homocysteine are quantified using gas chromatography–mass spectrometry (Møller and Rasmussen, Homocysteine in plasma: stabilization of blood samples with fluoride. Clin Chem 1995; 41:758–759).

Histologic Assessment of Atherosclerosis - After 28 weeks of diet feeding, mice are killed after anesthesia and blood collection as described elsewhere (Delsing et al, Acyl-coa: cholesterol acyltransferase inhibitor avasimibe reduces atherosclerosis in addition to its cholesterol-lowering effect in apoe*3-leiden mice. Circulation 2001; 103:1778–1786). The hearts are dissected, stored overnight in phosphate-buffered 3.8% formalin fixation, and embedded in paraffin. Serial cross-sections (5 µm thick, spaced 30 µm apart) throughout the entire aortic valve area are used for histologic analysis. Sections are routinely stained with hematoxylin-phloxine-saffron. Per mouse, 4 sections with intervals of 30 µm are used for quantification and qualification of atherosclerotic lesions. All sections are imaged and stored under identical lighting, microscopic (Nikon), camera (Hitachi), and computer conditions. Total lesion areas are determined using Leica Qwin image analysis software. The same operator, who is blinded to experimental group allocation, performs all analyses. The degree of calcification in the atherosclerotic lesions is determined by quantification of Von Kossa staining; collagen content is quantified morphometrically after staining with

Sirius Red. For determination of severity of atherosclerosis, the lesions are classified into five categories as described before (Delsing et al, 2001): [1] early fatty streak, [2] regular fatty streak, [3] mild plaque, [4] moderate plaque, and [5] severe plaque. Per mouse, the percentages of all lesions found in the respective lesion categories are calculated.

Statistical Analyses - Results are analyzed by 1-way ANOVA followed by application of the Tukey test to assess the significance of specific intergroup differences.

Example Five - Effect of P5P and Niacin Combination Therapy on Atherosclerosis in Mice

Objective - To investigate the anti-atherogenic effects of P5P in an animal model of atherosclerosis and to investigate the combination of P5P with niacin for enhanced or synergistic anti-atherogenic effect.

The study was designed to investigate the potential anti-atherogenic effects of P5P as compared with niacin and the combination of both in Apolipoprotein E-knockout (apoE-KO) mice. The study compares the effects of both drugs, alone and in combination, on atherosclerotic lesion formation, plasma lipoproteins, lipoprotein oxidation, homocysteine levels, and markers for inflammation.

Drugs - The drug niacin is available from Sigma. The dose of niacin chosen for the present study is based on the doses used in previous studies with hyperlipidemic mice (6). It is mixed in the diet of mice at a concentration of 1%. P5P is provided by CanAm Bioresearch Inc. The dose of 1 mg/kg body weight per day is also mixed into the diet of the mice.

Experimental Design -Male apoE-/- mice on a C57BL/6J background (n=60) are purchased from Jackson Laboratories (Bar Harbor, Me). Mice are fed a chow diet

and water ad libitum throughout the study. At 6 weeks of age, the animals are divided into four groups of 15 mice each, matched for their plasma lipid concentrations and body weight. The four groups of 15 mice are fed a PicoLab mouse diet (Jamieson's Pet Food Distributor) containing 9% (wt/wt) fat (controls), or the same diet supplemented with 1% niacin (niacin group), 1 mg/kg P5P (P5P group), or 1% niacin + 1 mg/kg P5P (niacin/P5P group). Animals are on the diets for 28 weeks, and are weighed biweekly. All animal experiments are approved by the institutional committee on animal welfare.

Lipid and Lipoprotein Analysis and Measurements of Fibrinogen, Serum Amyloid A, and von Willebrand Factor -After a 4-hour fasting period, blood samples are obtained by tail incision. Total plasma cholesterol, triglyceride levels, and lipoprotein profiles are measured at t = 0, 3, 6, 11, 19, 24, and 28 weeks as described elsewhere (Volger et al, Dietary plant stanol esters reduce VLDL cholesterol secretion and bile saturation in apolipoprotein e*3-leiden transgenic mice. Arterioscler Thromb Vasc Biol 2001; 21:1046–1052.). Plasma von Willebrand factor (vWF), serum amyloid A (SAA), and fibrinogen are measured by ELISA specific for vWF (Tranquille and Emeis, The simultaneous acute release of tissue-type plasminogen activator and von Willebrand factor in the perfused rat hindleg region. Thromb Haemost 1990; 63:454–458.), SAA (Biosource), and fibrinogen (Kockx et al, Fibric acid derivatives suppress fibrinogen gene expression in rodents via activation of the peroxisome proliferator-activated receptor-[alpha]. Blood 1999; 93:2991–2998).

Plasma Oxidized LDL Concentrations - Ninety-six–well polystyrene plates (Nunc) are coated with either oxidized LDL, at a concentration of 10 µg/mL in PBS, or native LDL (both from humans) overnight at 4°C. The subsequent steps are performed as described previously (10). IgG isotypes are determined with an ELISA kit (Southern Biotechnology).

Plasma Homocysteine Concentrations - Nonfasting blood samples are obtained and plasma homocysteine is quantified using gas chromatography–mass spectrometry (Møller and Rasmussen, Homocysteine in plasma: stabilization of blood samples with fluoride. Clin Chem 1995; 41:758–759.).

Histologic Assessment of Atherosclerosis- After 28 weeks of diet feeding, the mice are killed after anesthesia and blood collected as described elsewhere (Delsing et al, Acyl-coa: cholesterol acyltransferase inhibitor avasimibe reduces atherosclerosis in addition to its cholesterol-lowering effect in apoe*3-leiden mice. Circulation 2001; 103:1778–1786). The hearts are dissected, stored overnight in phosphate-buffered 3.8% formalin fixation, and embedded in paraffin. Serial cross-sections (5 µm thick, spaced 30 µm apart) throughout the entire aortic valve area are used for histologic analysis. Sections will be routinely stained with hematoxylin-phloxine-saffron. Per mouse, 4 sections with intervals of 30 µm are used for quantification and qualification of atherosclerotic lesions. All sections are imaged and stored under identical lighting, microscopic (Nikon), camera (Hitachi), and computer conditions. Total lesion areas will be determined using Leica Qwin image analysis software. The same operator, who is blinded to experimental group allocation, performs all analyses. The degree of calcification in the atherosclerotic lesions is determined by quantification of Von Kossa staining; collagen content is quantified morphometrically after staining with Sirius Red. For determination of severity of atherosclerosis, the lesions are classified into five categories as described before (Delsing et al, 2001): [1] early fatty streak, [2] regular fatty streak, [3] mild plaque, [4] moderate plaque, and [5] severe plaque. Per mouse, the percentages of all lesions found in the respective lesion categories are calculated.

Statistical Analyses - Results are analyzed by 1-way ANOVA followed by application of the Tukey test to assess the significance of specific intergroup differences.

CLAIMS

What is claimed is:

1. A pharmaceutical composition comprising: (a) a nicotinic acid derivative or a fibric acid derivative; (b) pyridoxal-5'-phosphate or pyridoxal-5'-phosphate related compound; and (c) a pharmaceutically acceptable carrier.

2. The pharmaceutical composition according to claim 1 wherein the fibric acid derivative is selected from a group consisting of: bezafibrate, clofibrate, ciprofibrate, fenofibrate, and gemfibrozil, and a mixture thereof.

3. The pharmaceutical composition according to claim 1 wherein the nicotinic acid derivative is selected from a group consisting of niacin, niceritrol, acipimox, and acifran.

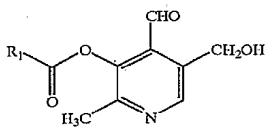
4. The pharmaceutical composition according to claim 1 wherein the pyridoxal-5'-phosphate or pyridoxal-5'-phosphate related compound is selected from a group consisting: pyridoxal, pyridoxal-5'-phosphate, pyridoxamine, a 3-acylated analogue of pyridoxal, a 3-acylated analogue of pyridoxal-4,5-aminal, a pyridoxine phosphate analogue, and a mixture thereof.

5. The pharmaceutical composition according to claim 1 wherein the pyridoxal-5'-phosphate or pyridoxal-5'-phosphate related compound is pyridoxal-5-phosphate.

6. The pharmaceutical composition according to claim 1 wherein the nicotinic acid derivative is niacin.

7. The pharmaceutical composition according to claim 1 wherein the fibric acid derivative is fenofibrate.

8. The pharmaceutical composition according to claim 4 wherein the 3-acylated analogue of pyridoxal is:



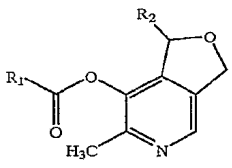
wherein,

R_1 is alkyl, alkenyl, in which alkyl can interrupted by nitrogen, oxygen, or sulfur, and can be unsubstituted or substituted at the terminal carbon with hydroxy, alkoxy, alkanoyloxy, alkoxyalkanoyl, alkoxycarbonyl, or

R_1 is dialkylcarbamoyloxy; alkoxy; dialkylamino; alkanoyloxy; alkanoyloxyaryl; alkoxyalkanoyl; alkoxycarbonyl; dialkylcarbamoyloxy; or

R_1 is aryl, aryloxy, arylthio, or aralkyl, in which aryl can be substituted by alkyl, alkoxy, amino, hydroxy, halo, nitro, or alkanoyloxy;

9. The pharmaceutical composition according to claim 4 wherein the 3-acylated analogue of pyridoxal-4,5-aminal is



wherein,

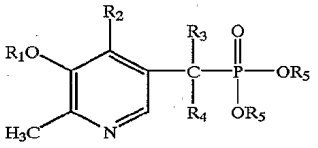
R_1 is alkyl, alkenyl, in which alkyl can interrupted by nitrogen, oxygen, or sulfur, and can be unsubstituted or substituted at the terminal carbon with hydroxy, alkoxy, alkanoyloxy, alkoxyalkanoyl, alkoxycarbonyl, or

R_1 is dialkylcarbamoyloxy; alkoxy; dialkylamino; alkanoyloxy; alkanoyloxyaryl; alkoxyalkanoyl; alkoxycarbonyl; dialkylcarbamoyloxy; or

R_1 is aryl, aryloxy, arylthio, or aralkyl, in which aryl can be substituted by alkyl, alkoxy, amino, hydroxy, halo, nitro, or alkanoyloxy;

R_2 is a secondary amino group.

10. The pharmaceutical composition according to claim 4 wherein the pyridoxine phosphate analogue is selected from a group consisting:



(a)

wherein,

R_1 is hydrogen or alkyl;

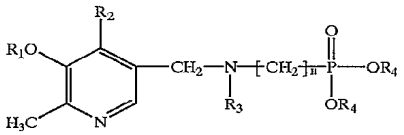
R_2 is –CHO-, -CH_2OH, -CH_3, -CO_2R6 in which R6 is hydrogen, alkyl, aryl; or

R_2 is –CH_2-O alkyl in which alkyl is covalently bonded to the oxygen at the 3-position instead of R_1;

R_3 is hydrogen and R_4 is hydroxy, halo, alkoxy, alkanoyloxy, alkylamino, or arylamino; or

R_3 and R_4 are halo; and

R_5 is hydrogen, alkyl, aryl, aralkyl, or $-CO_2R_7$ in which R_7 is hydrogen, alkyl, aryl, or aralkyl;



(b)

wherein,

R_1 is hydrogen or alkyl;

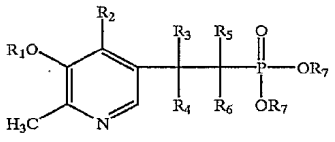
R_2 is –CHO, $-CH_2OH$, $-CH_3$, $-CO_2R_5$ in which R_5 is hydrogen, alkyl, aryl; or

R_2 is $-CH_2$-O alkyl in which alkyl is covalently bonded to the oxygen at the 3-position instead of R_1;

R_3 is hydrogen, alkyl, aryl, aralkyl,

R_4 is hydrogen, alkyl, aryl, aralkyl, or $-CO_2R6$ in which R6 is hydrogen, alkyl, aryl or aralkyl;

n is 1 to 6; and



(c)

wherein,

R_1 is hydrogen or alkyl;

R_2 is –CHO-, CH_2OH-, -CH_3, -CO_2R_8 in which R_8 is hydrogen, alkyl, aryl; or

R_2 is –CH_2-O alkyl- in which alkyl is covalently bonded to the oxygen at the 3-position instead of R_1;

R_3 is hydrogen and R_4 is hydroxy, halo, alkoxy, or alkanoyloxy; or

R_3 and R_4 can be taken together to form =O;

R_5 and R6 are hydrogen; or

R_5 and R6 are halo;

R_7 is hydrogen, alkyl, aryl, aralkyl, or –CO_2R_8 in which R_8 is hydrogen, alkyl, aryl, or aralkyl.

11. A method for treating or preventing cardiovascular disease in a patient comprising administering a therapeutically effective dose of the pharmaceutical composition according to any one of claims 1 to 10.

12. The method according to claim 11, wherein the patient is susceptible to hepatotoxicity.

13. The method according to claim 11 wherein the cardiovascular disease is selected from a group consisting: congestive heart failure, myocardial ischemia, arrhythmia, myocardial infarction, ischemic stroke, hemorrhagic stroke, coronary artery disease, hypertension (high blood pressure), atherosclerosis (clogging of the arteries), aneurysm, peripheral artery disease (PAD), thrombophlebitis (vein inflammation), diseases of the heart lining, diseases of the heart muscle, carditis, congestive heart failure, endocarditis, ischemic heart disease, valvular heart disease

(malfunction of a valve or valves in the blood vessels of the heart), arteriosclerosis (hardening of the arteries), acute coronary syndrome (ACS), deep vein thrombosis (DVT), Kawazaki disease, high cholesterol, and heart transplant.

14. The method according to claim 11 wherein the dose of the nicotinic acid derivative is between 0.1 and 5000 mg per day.

15. The method according to claim 11 wherein the dose of the nicotinic acid derivative is between 100 and 3000 mg per day.

16. The method according to claim 11 wherein the dose of the nicotinic acid derivative is selected from the group consisting of 100, 250, 500, 1000 and 3000 mg per day.

17. The method according to claim 11 wherein the dose of the fibric acid derivative is between 0.1 and 1000 mg per day.

18. The method according to claim 11 wherein the dose of the fibric acid derivative is selected from the group consisting of 100, 200, 400 and 600 mg per day.

19. The method according to claim 11 wherein the fibric acid derivative is bezafibrate and the dose is between 400 and 600 mg per day.

20. The method according to claim 11 wherein the fibric acid derivative is ciprofibrate and the dose is 200 mg per day.

21. The method according to claim 11 wherein the fibric acid derivative is gemfibrozil and the dose is between 400 and 600 mg per day.

22. The method according to claim 11 wherein the fibric acid derivative is fenofibrate and the dose is between 40 and 200 mg per day.

23. The method according to claim 11 wherein the dose of the pyridoxal-5'-phosphate or pyridoxal-5'-phosphate related compound is between 0.1 to 50 mg/kg per day.

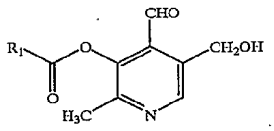
24. The method according to claim 11 wherein the dose of pyridoxal-5'-phosphate or pyridoxal-5'-phosphate related compound is between 1 to 15 mg/kg per day.

25. A method of treating or preventing diabetes comprising administering a therapeutically effective dose of the pharmaceutical composition according to any one of claims 1 to 10.

26. A method of treating or preventing hypercholesterolemia in a patient, comprising administering a therapeutically effective dose of: (a) a nicotinic acid derivative or a fibric acid derivative and (b) a pyridoxal-5'-phosphate or pyridoxal-5'-phosphate related compound wherein the pyridoxal-5'-phosphate or pyridoxal-5'-phosphate related compound is selected from a group consisting: pyridoxal-5'-phosphate, a 3-acylated analogue of pyridoxal, a 3-acylated analogue of pyridoxal-4,5-aminal, a pyridoxine phosphate analogue, and a mixture thereof.

27. The method according to claim 26 wherein the pyridoxal-5'-phosphate or pyridoxal-5'-phosphate related compound is pyridoxal-5-phosphate.

28. The method according to claim 26 wherein the 3-acylated analogue of pyridoxal is:



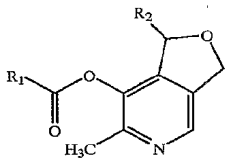
wherein,

R_1 is alkyl, alkenyl, in which alkyl can interrupted by nitrogen, oxygen, or sulfur, and can be unsubstituted or substituted at the terminal carbon with hydroxy, alkoxy, alkanoyloxy, alkoxyalkanoyl, alkoxycarbonyl, or

R_1 is dialkylcarbamoyloxy; alkoxy; dialkylamino; alkanoyloxy; alkanoyloxyaryl; alkoxyalkanoyl; alkoxycarbonyl; dialkylcarbamoyloxy; or

R_1 is aryl, aryloxy, arylthio, or aralkyl, in which aryl can be substituted by alkyl, alkoxy, amino, hydroxy, halo, nitro, or alkanoyloxy;

29. The method according to claim 26 wherein the 3-acylated analogue of pyridoxal-4,5-aminal is



wherein,

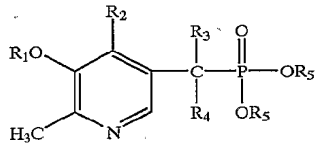
R_1 is alkyl, alkenyl, in which alkyl can interrupted by nitrogen, oxygen, or sulfur, and can be unsubstituted or substituted at the terminal carbon with hydroxy, alkoxy, alkanoyloxy, alkoxyalkanoyl, alkoxycarbonyl, or

R_1 is dialkylcarbamoyloxy; alkoxy; dialkylamino; alkanoyloxy; alkanoyloxyaryl; alkoxyalkanoyl; alkoxycarbonyl; dialkylcarbamoyloxy; or

R_1 is aryl, aryloxy, arylthio, or aralkyl, in which aryl can be substituted by alkyl, alkoxy, amino, hydroxy, halo, nitro, or alkanoyloxy;

R_2 is a secondary amino group.

30. The pharmaceutical composition according to claim 26 wherein the pyridoxine phosphate analogue is selected from a group consisting:



(a)

wherein,

R_1 is hydrogen or alkyl;

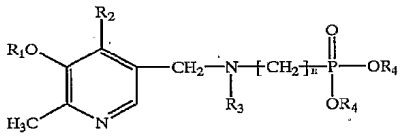
R_2 is –CHO-, -CH_2OH, -CH_3, -CO_2R6 in which R6 is hydrogen, alkyl, aryl; or

R_2 is –CH_2-O alkyl in which alkyl is covalently bonded to the oxygen at the 3-position instead of R_1;

R_3 is hydrogen and R_4 is hydroxy, halo, alkoxy, alkanoyloxy, alkylamino, or arylamino; or

R_3 and R_4 are halo; and

R_5 is hydrogen, alkyl, aryl, aralkyl, or –CO_2R_7 in which R_7 is hydrogen, alkyl, aryl, or aralkyl;



(b)

wherein,

R_1 is hydrogen or alkyl;

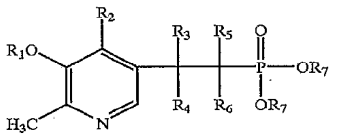
R_2 is –CHO, -CH_2OH, -CH_3, -CO_2R_5 in which R_5 is hydrogen, alkyl, aryl; or

R_2 is –CH_2-O alkyl in which alkyl is covalently bonded to the oxygen at the 3-position instead of R_1;

R_3 is hydrogen, alkyl, aryl, aralkyl,

R_4 is hydrogen, alkyl, aryl, aralkyl, or -CO_2R6 in which R6 is hydrogen, alkyl, aryl or aralkyl;

n is 1 to 6; and

(c) 

wherein,

R_1 is hydrogen or alkyl;

R_2 is –CHO-, CH_2OH-, -CH_3, -CO_2R_8 in which R_8 is hydrogen, alkyl, aryl; or

R_2 is –CH_2-O alkyl- in which alkyl is covalently bonded to the oxygen at the 3-position instead of R_1;

R_3 is hydrogen and R_4 is hydroxy, halo, alkoxy, or alkanoyloxy; or

R_3 and R_4 can be taken together to form =O;

R_5 and R6 are hydrogen; or

R_5 and R6 are halo;

R_7 is hydrogen, alkyl, aryl, aralkyl, or $-CO_2R_8$ in which R_8 is hydrogen, alkyl, aryl, or aralkyl.

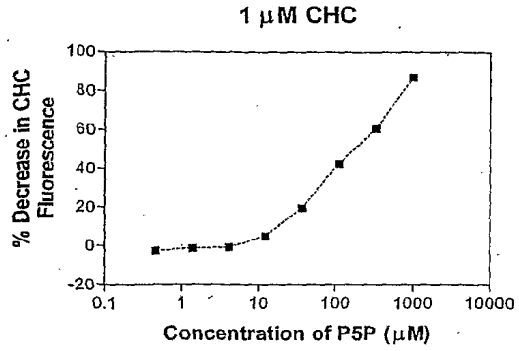
31. Use of a pyridoxal-5'-phosphate or pyridoxal-5'-phosphate related compound to decrease the side effects of nicotinic acid derivative administration.

32. The use as claimed in claim 31 wherein the nicotinic acid derivative is niacin.

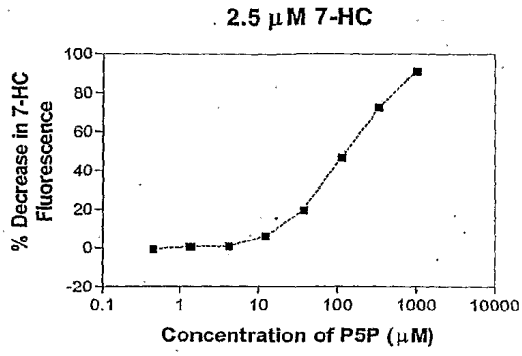
33. The use as claimed in claim 31 wherein the side effect is selected from a group consisting of an elevated homocysteine level and an elevated thromboxane A2 level.

34. The use as claimed in claim 31 wherein the pyridoxal-5'-phosphate or pyridoxal-5'-phosphate related compound is pyridoxal-5'-phosphate.

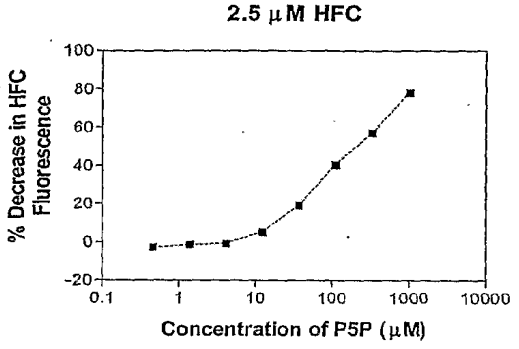
FIGURE 1



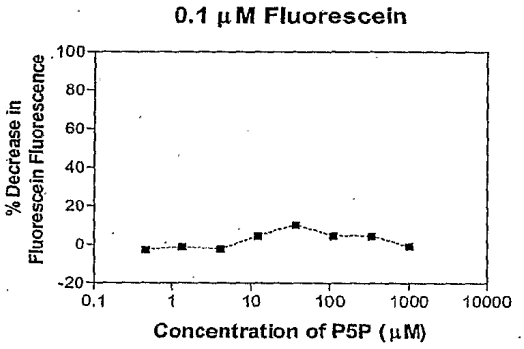
1(a)



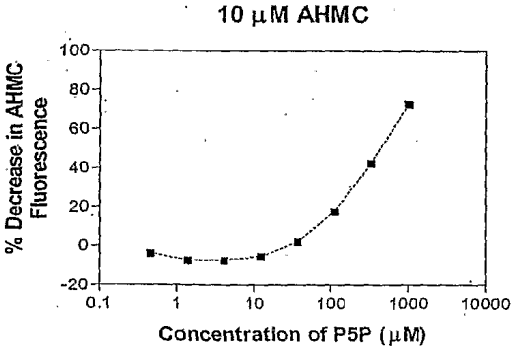
1(b)



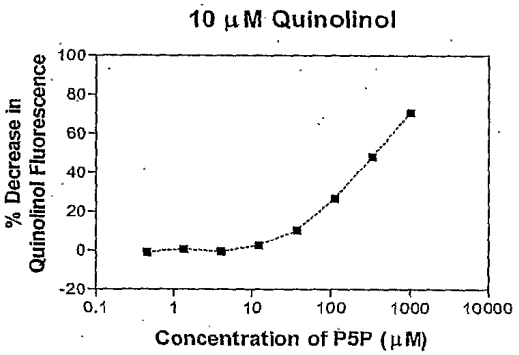
1(c)



1(d)

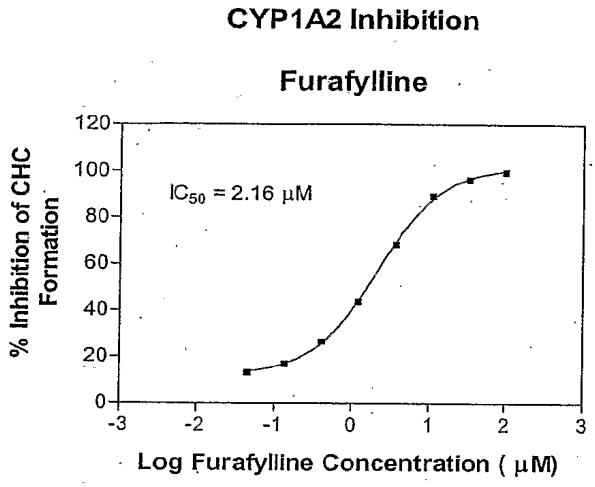


1(e)

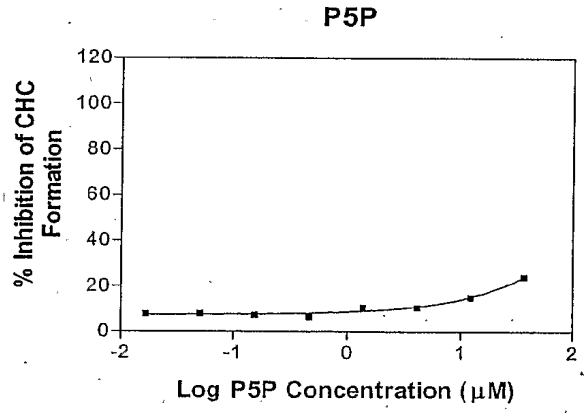


1(f)

FIGURE 2

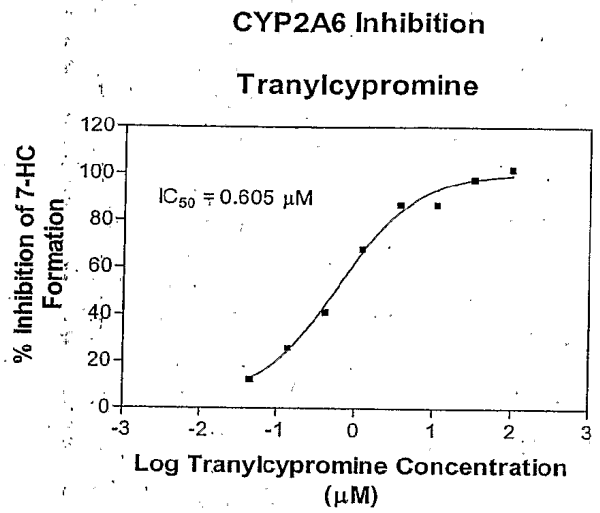


2(a)

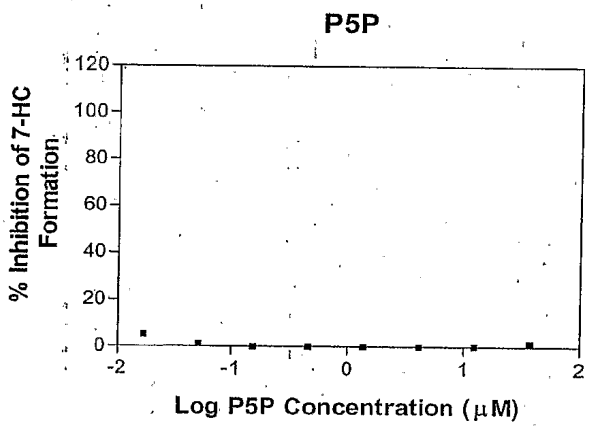


2(b)

FIGURE 3

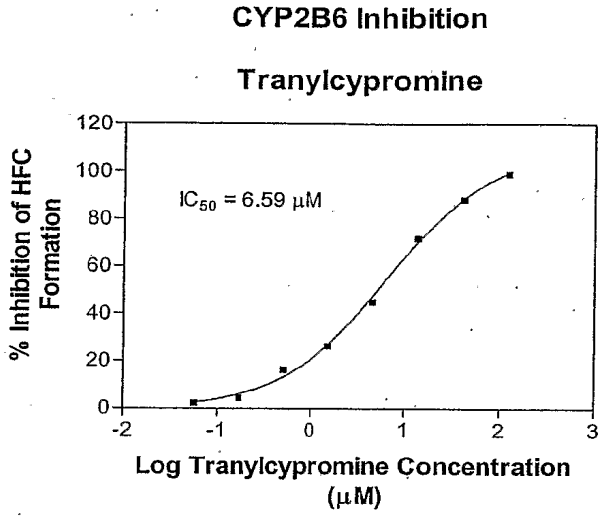


3(a)

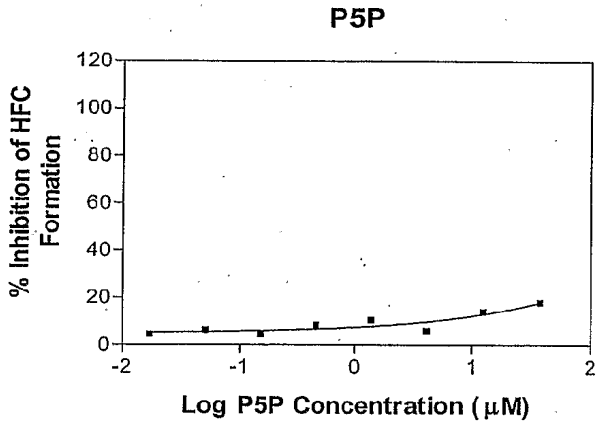


3(b)

FIGURE 4

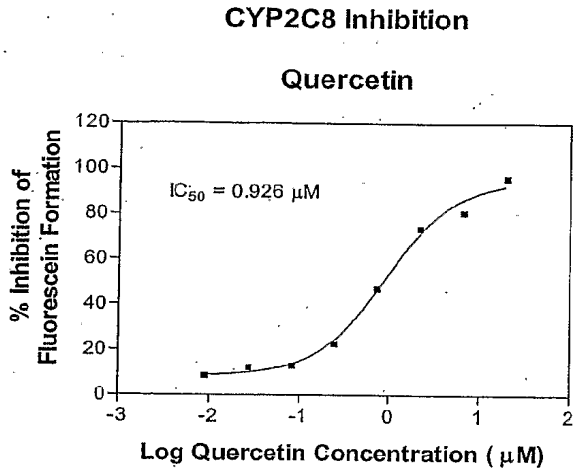


4(a)

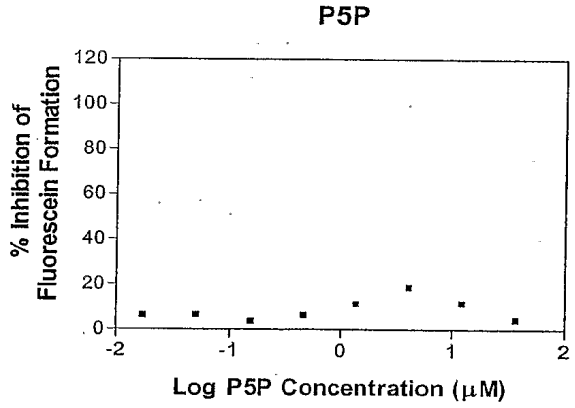


4(b)

FIGURE 5

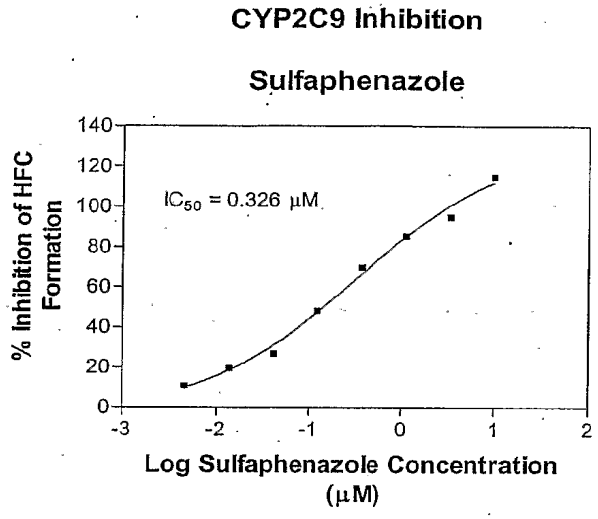


5(a)

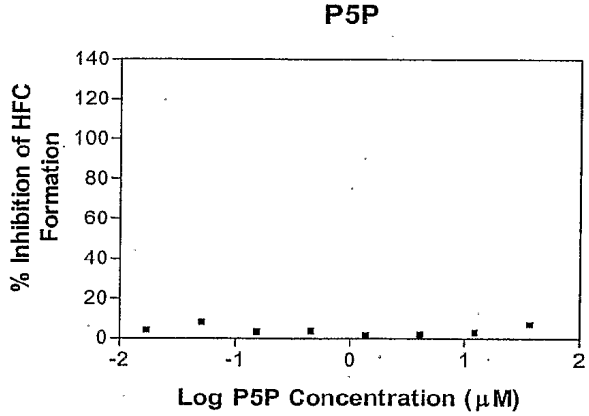


5(b)

FIGURE 6

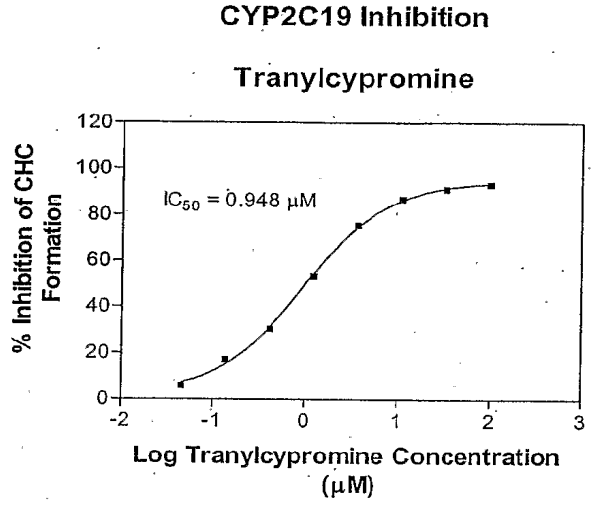


6(a)

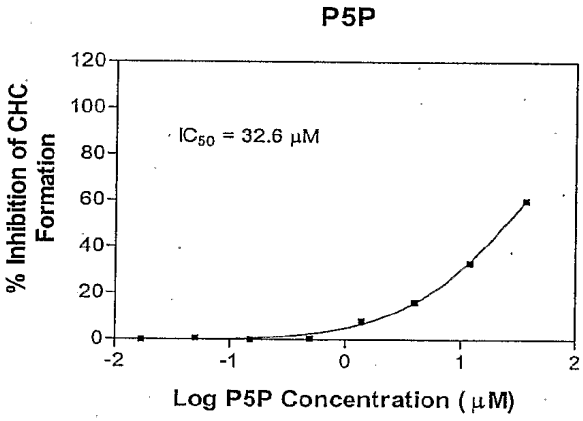


6(b)

FIGURE 7

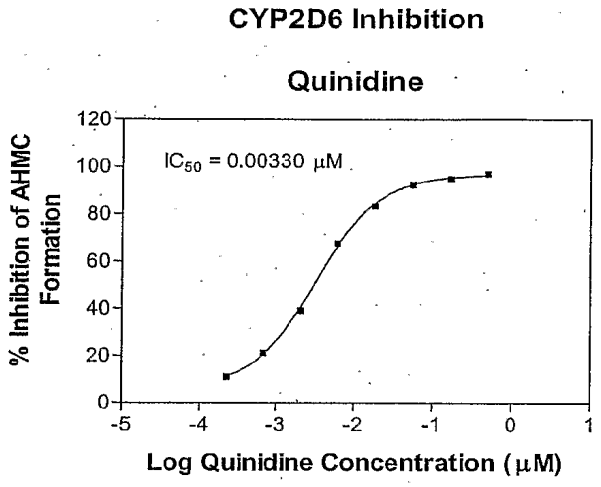


7(a)

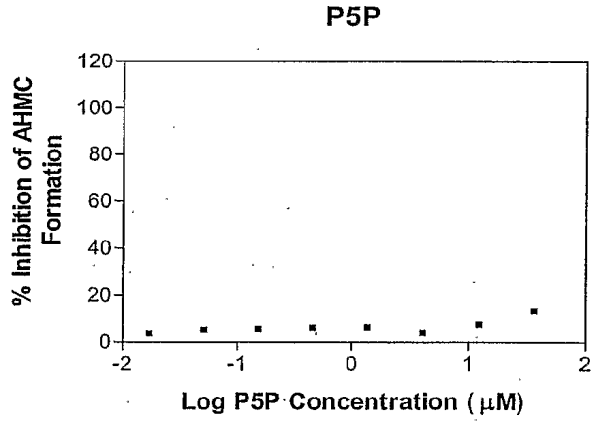


7(b)

FIGURE 8

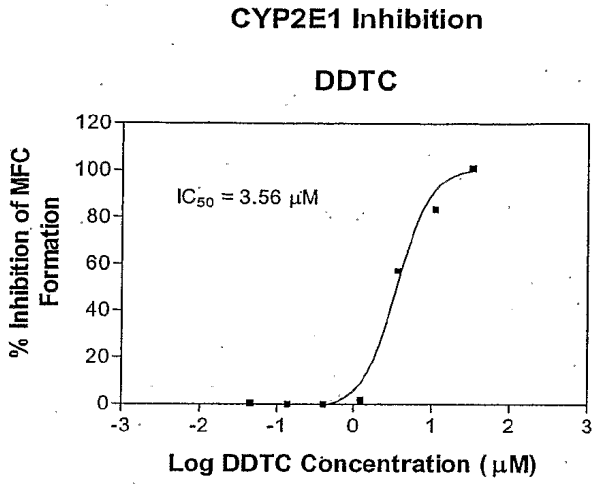


8(a)

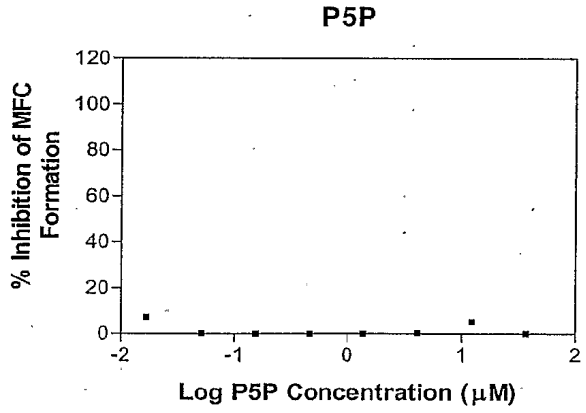


8(b)

FIGURE 9

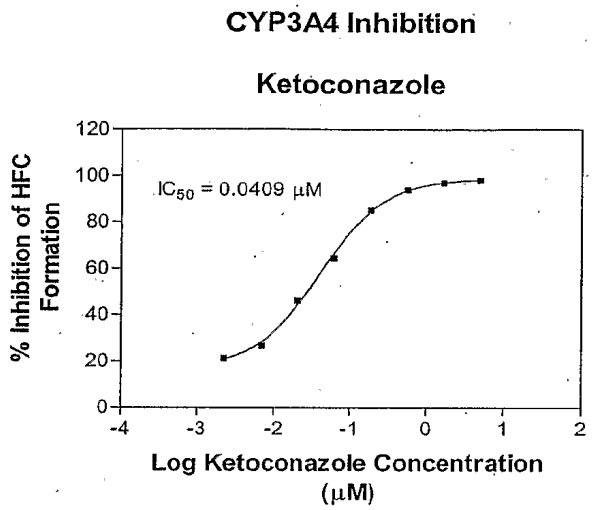


9(a)

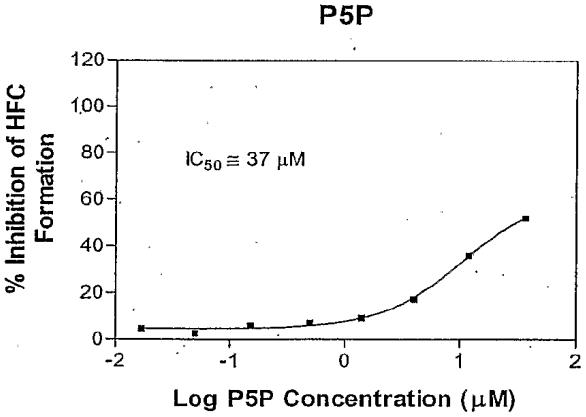


9(b)

FIGURE 10

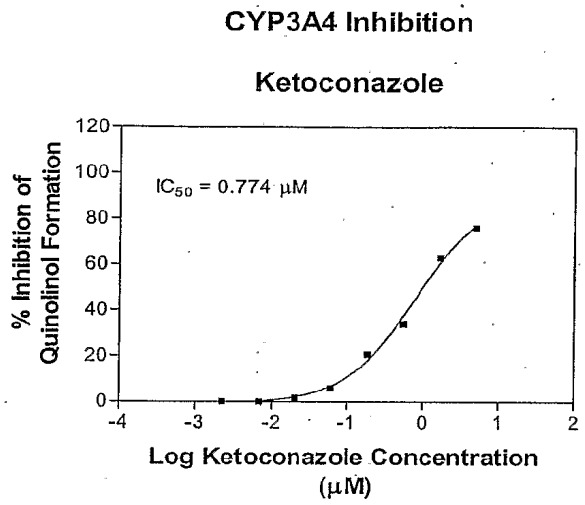


10(a)

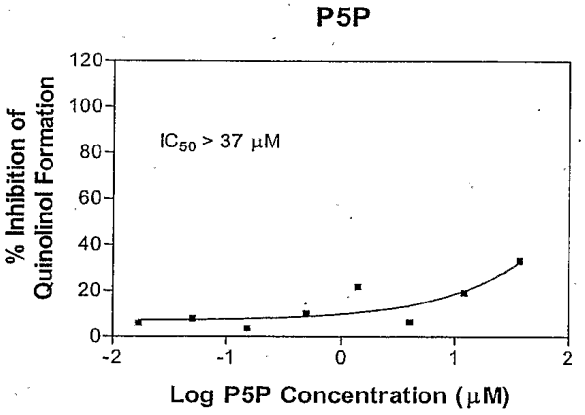


10(b)

FIGURE 11



11(a)



11(b)

FIGURE 12

CYP Subtype	Substrate	Inhibitor (positive control)	Expected Inhibitor IC_{50}	Observed Inhibitor IC_{50}	Pyridoxal 5'-phosphate IC_{50}
			(μM)		
CYP1A2	CEC	Furafylline	2.22 ± 1.15[a]	2.16	na
CYP2A6	Coumarin	Tranylcypromine	0.990 ± 0.209[a]	0.605	na
CYP2B6	EFC	Tranylcypromine	8.82 ± 2.43[a]	6.59	na
CYP2C8	DBF	Quercetin	1.40 ± 0.240[a]	0.926	na
CYP2C9	MFC	Sulfaphenazole	0.401 ± 0.116[a]	0.326	na
CYP2C19	CEC	Tranylcypromine	0.825[b]	0.948	32.6
CYP2D6	AMMC	Quinidine	0.00544 ± 0.00173[a]	0.00330	na
CYP2E1	MFC	DDTC	5.06 ± 1.96[a]	3.56	na
CYP3A4	BFC	Ketoconazole	0.018[b]	0.0409	≅37
	BQ		0.400[b]	0.774	>37

[a] value expected from assay validation (mean ± S.D., n ≥8 experiments).
[b] value determined by the supplier using all the components contained in the current lot kit.
na denotes not applicable since concentration-dependent inhibition was not observed over the concentration range tested.

FIGURE 13

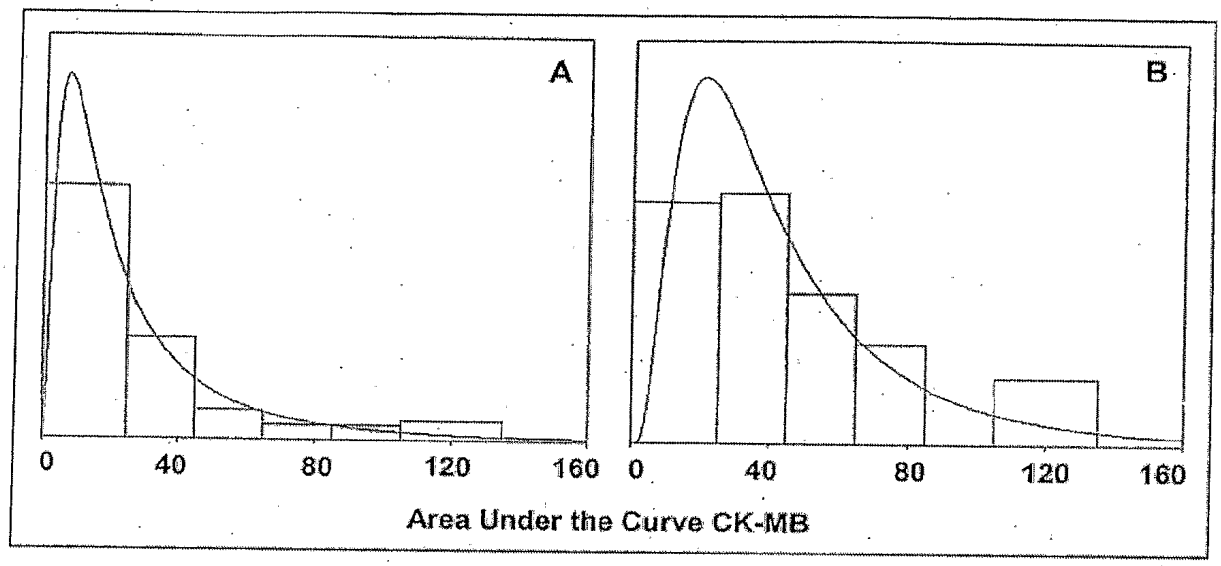


FIGURE 14

TABLE 1 Baseline Clinical, Electrocardiographic, and Angiographic Characteristics

	M P5P 10]	Placebo (n = 20)
Clinical characteristics*		
Age (yrs) (range)	54 (48–66)	59 (55–69)
Men	32 (80)	17 (85)
Baseline troponin positive	14/30 (47)	6/14 (43)
Diabetes mellitus	9 (23)	4 (20)
Systemic hypertension	17 (43)	9 (45)
Hyperlipidemia (requiring medical treatment or LDL >130 mg/dl)	31 (78)	17 (85)
Current smoker	12 (30)	5 (25)
Prior myocardial infarction	14 (35)	9 (45)
Prior PCI	6 (15)	2 (10)
Prior coronary bypass graft surgery	5 (13)	2 (10)
Prior stroke or transient ischemic attack	1 (3)	1 (5)
Peripheral vascular disease	3 (8)	7 (35)
Congestive heart failure	3 (8)	2 (10)
Qualifying electrocardiogram		
ST-segment depression	2 (5)	2 (10)
ST-segment elevation	7 (18)	2 (10)
T-wave inversion	6 (15)	4 (20)
Angiographic characteristics		
PCI performed	37 (93)	19 (95)
Reason for PCI	(n = 37)	(n = 19)
Acute coronary syndrome	9 (24)	5 (25)
Recent AMI	16 (42)	3 (15)
Reduced epicardial flow	6 (16)	8 (40)
Thrombus	1 (3)	1 (5)
Congestive heart failure	2 (5)	1 (5)
Saphenous vein graft lesion	4 (11)	2 (10)
No. of coronary arteries narrowed ≥50% in diameter	(n = 37)	(n = 19)
0	1 (3)	0
1	19 (48)	14 (70)
2	13 (33)	2 (10)
3	5 (13)	3 (15)
Left main	2 (5)	1 (5)
Left ventricular ejection fraction	0.50 (0.40–0.68)	0.56 (0.37–0.64)
No. of coronary narrowings treated	(n = 37)	(n = 19)
1	26 (70)	15 (79)
2	8 (22)	3 (16)
3	3 (8)	1 (5)

Values are expressed as median (interquartile range) or number (percent).
*Patients may be double counted.
LDL = low-density lipoprotein.

FIGURE 15

TABLE 2 Procedural and Angiographic* results

	P5P (n = 37)	Placebo (n = 19)
≥1 stent implanted	36 (97)	19 (100)
Patients received GP IIb/IIIa inhibitor	29/35 (83)	15/19 (79)
Target vessel		
Left anterior descending	11 (30)	4 (21)
Right	14 (38)	11 (58)
Left circumflex	8 (22)	3 (16)
Saphenous vein graft	4 (11)	1 (5)
TIMI flow preprocedure		
0/1	3 (8)	4 (22)
2	7 (19)	4 (22)
3	27 (73)	10 (56)
TIMI flow final		
0/1	0	0
2	0	1 (5)
3	37 (100)	18 (95)
Diameter stenosis preprocedure (%)	90.0 (80.0–95.0)	95.0 (90.0–99.0)
Diameter stenosis final (%)	0 (0–0)	0 (0–0)
Procedural complications		
None	35 (95)	18 (95)
Major dissection	1 (3)	1 (5)
Abrupt closure	0	0
No reflow	0	0
Thrombus formation	0	0
Side branch closure	1 (3)	0
Distal embolization	0	0

Values are expressed as median (interquartile range) or number (percent).
*Investigator-reported angiographic values.
GP = glycoprotein.

FIGURE 16

TABLE 3 Periprocedural Cardiac Markers and ST Monitoring Results

	P5P	Placebo	p Value
Periprocedural cardiac markers			
Area under the curve CK-MB (ng/ml)	18.6 (10.2–34.5), 35	32.9 (19.4–64.3), 18	0.04
Peak CK-MB (ng/ml)	1.1 (0.5–2.4), 39	2.0 (1.4–6.3), 19	0.03
Change in troponin T (ng/ml)	0 (0–0.07), 36	0 (0–0.10), 19	0.65
Time to peak CK-MB (h)	11.0 (0–18.0), 36	14.0 (12.0–18.0), 19	0.10
24-h continuous electrocardiographic ST monitoring			
Duration of monitoring (h)	22.6 (20.4–23.9), 36	22.4 (20.6–24.0), 17	—
Area under the curve ST deviation (μV-min)	1349 (951–2,263), 35	1603 (1,049–1,945), 17	0.49
Any post-PCI ischemia (%)	14.7–34	17.6–17	0.78

Values are expressed as median (interquartile range) or percent followed by n (number of observations).

INTERNATIONAL SEARCH REPORT

International application No.
PCT/CA2005/001070

A. CLASSIFICATION OF SUBJECT MATTER
IPC(7): A61K 31/675, A61K 31/455, A61K 31/216, A61P 7/00, A61P 3/06

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC[7] A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic database(s) consulted during the international search (name of database(s) and, where practicable, search terms used)
Delphion, PubMed, Canadian Patent Database; *keywords*: niacin, niceritrol, acipimox, acifran, vitamin B6, cholesterol, cardiovascular, combination therapy, PLP, pyridoxal-5'-phosphate, pyridoxine, pyridoxal, pyridoxamine

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	The Journal of Nutrition, Vol. 127, No. 1, January 1997, BASU ET AL., "Vitamin B-6 Normalizes the Altered Sulfur Amino Acid Status of Rats Fed Diets Containing Pharmacological Levels of Niacin without Reducing Niacin's Hypolipidemic Effects", pages 117-121 * see abstract, page 120*	1 (part), 3, 4-5 (part), 6, 8-13 (part), 14-16, 23-24 (part), 26-30 (part)
Y	American Journal of Health-System Pharmacists, Vol. 60, Suppl.2, July 1 2003, ITO, "Advances in the understanding and management of Dyslipidemia: Using niacin-based therapies", S15-S21 *see pages S18-S20*	1 (part), 3, 4-5 (part), 6, 8-13 (part), 14-16, 23-24 (part), 26-30 (part)
Y	WO 01/56609 (WESTPHAL ET AL.) 9 August 2001 (09-08-2001) *see entire application*	1 (part), 3, 4-5 (part), 6, 8-13 (part), 14-16, 23-24 (part), 26-30 (part)

[X] Further documents are listed in the continuation of Box C.

[X] See patent family annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
13 October 2005 (13-10-2005)	14 October 2005 (14-10-2005)

Name and mailing address of the ISA/CA	Authorized officer
Canadian Intellectual Property Office Place du Portage I, C114 - 1st Floor, Box PCT 50 Victoria Street Gatineau, Quebec K1A 0C9 Facsimile No.: 001(819)953-2476	Stephanie Michaud (819) 934-2328

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	WO 00/57863 (DHALLA ET AL.) 5 October 2000 (05-10-2000) *see entire application*	1 (part), 3, 4-5 (part), 6, 8-13 (part), 14-16, 23-24 (part), 26-30 (part)
Y	WO 00/53606 (HAQUE) 14 September 2000 (14-09-2000) *see entire application*	1 (part), 3, 4-5 (part), 6, 8-13 (part), 14-16, 23-24 (part), 26-30 (part)
Y	WO 01/64692 (HAQUE) 7 September 2001 (07-09-2001) *see entire application*	1 (part), 3, 4-5 (part), 6, 8-13 (part), 14-16, 23-24 (part), 26-30 (part)
Y	Hypertension, Vol 38, No. 5, November 2001, GARCIA-TEVIJIANO ET AL., "Hyperhomocysteinemia in Liver Cirrhosis: Mechanisms and Role in Vascular and Hepatic Fibrosis", pages 1217-1221 *see abstract, pages 1220-1221*	1 (part), 3, 4-5 (part), 6, 8-13 (part), 14-16, 23-24 (part), 26-30 (part)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/CA2005/001070

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of the first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons :

1. [X] Claim Nos. : 11-34
because they relate to subject matter not required to be searched by this Authority, namely :

Although claims 11-34 are directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition as described on pages 3, 8-9 of the description.

2. [X] Claim Nos. : 1-34 (in part)
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically :

see PCT/ISA/210 (Extra Sheet)

3. [ ] Claim Nos. :
because they are dependant claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows :

see PCT/ISA/210 (Extra Sheet)

1. [ ] As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. [ ] As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claim Nos. :

4. [X] No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claim Nos. :

Group A: Claims 1 (part), 3, 4-5 (part), 6, 8-13 (part), 14-16, 23-24 (part), 26-30 (part)

Remark on Protest [ ] The additional search fees were accompanied by the applicant's protest and, where the payment of a protest fee.

[ ] The additional search fees were accompanied by the applicant's protest but the applicable fee was not paid within the time limit specified in the invitation.

[ ] No protest accompanied the payment of additional search fees.

Continuation of **Box No. II Part 2.**

Claims 1-34 (in part):

Claims 1, 26, 31 do not meet the requirements of Article 6 PCT because the subject-matter is defined in terms of the result to be achieved rather than in terms of technical features, as required by Rule 6.3(a) PCT. Thus, the following expression is considered to be a functional feature:

"pyridoxal-5'-phosphate or pyridoxal-5'-phosphate related compound".

Since the components of the pharmaceutical composition of claim 1 and use thereof in the method of claims 26, 31 are defined solely by reference to desirable characteristics, namely as "pyridoxal-5'-phosphate or pyridoxal-5'-phosphate *related* compound" a meaningful search over the whole of the claimed scope within the meaning of Article 6 PCT for claims 1, 26, 31 is impossible. Furthermore, the use of the expressions "derivative" (claim 1) and "analogue" (claim 4) in the present context is also considered to lead to a lack of clarity within the meaning of Article 6. The lack of clarity with respect to the functional claiming and ambiguous definitions is such as to render a meaningful and complete search impossible. Due to the multiple meanings that can be arrived at for "pyridoxal-5'-phosphate or pyridoxal-5'-phosphate related compound"; "nicotinic acid derivative", "fibric acid derivative"; "3-acylated analogue of pyridoxal"; "3-acylated analogue of pyridoxal-4,5-aminal", and "pyridoxine phosphate analogue", a complete prior art search was precluded and limited to those components of the composition that appear to be supported. Therefore, the search was limited to those pharmaceutical compositions and method of use therein comprising the nicotinic acid derivatives defined in claim 3 and the pyridoxal-5'-phosphate or pyridoxal-5'-phosphate related compounds: pyridoxal, pyridoxal-5'-phosphate, the 3-acylated analogues of pyridoxal defined in claim 8, the 3-acylated analogues of pyridoxal-4,5-aminal defined in claim 9, and the pyridoxine phosphate analogues defined in claim 10, for claims 1-34.

Continuation of **Box No. III**

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

Group A: Claims 1 (part), 3, 4-5 (part), 6, 8-13 (part), 14-16, 23-24 (part), 26-30 (part) are directed to a pharmaceutical composition comprising a nicotinic acid derivative and pyridoxal-5'-phosphate or a pyridoxal-5'-phosphate related compound, and methods of use therein for treating or preventing cardiovascular disease or hypercholesterolemia.

Group B: Claims 1 (part), 2, 4-5 (part), 7, 8-13 (part), 17-22, 23-24 (part), 26-30 (part) are directed to a pharmaceutical composition comprising a fibric acid derivative and pyridoxal-5'-phosphate or a pyridoxal-5'-phosphate related compound, and methods of use therein for treating or preventing cardiovascular disease or hypercholesterolemia.

Group C: Claim 25 (part) is directed to a method of treating or preventing diabetes comprising administering a pharmaceutical composition comprising a fibric acid derivative and pyridoxal-5'-phosphate or a pyridoxal-5'-phosphate related compound.

Group D: Claim 25 (part) is directed to a method of treating or preventing diabetes comprising administering a pharmaceutical composition comprising a nicotinic acid derivative and pyridoxal-5'-phosphate or a pyridoxal-5'-phosphate related compound.

Group E: Claims 31-34 are directed to the use of pyridoxal-5'-phosphate or a pyridoxal-5'-phosphate related compound to decrease the side effects of nicotinic acid derivative administration.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No.
PCT/CA2005/001070

Patent Document Cited in Search Report	Publication Date	Patent Family Member(s)	Publication Date
WO0156609	09-08-2001	AU7285800 A	14-08-2001
		DE10004651 A1	16-08-2001
WO0057863	5-10-2000	AT259643T T	15-03-2004
		AU759824 B2	01-05-2003
		AU779238 B2	13-01-2005
		AU3314700 A	16-10-2000
		AU9421098 A	03-02-2000
		BR0009292 A	26-12-2001
		CA2254528 A1	09-01-2000
		CA2368775 A1	05-10-2000
		CN1208922 A	24-02-1999
		DE60008352D	25-03-2004
		DE60008352T	23-12-2004
		DK1162980T T3	14-06-2004
		EP1162980 A2	19-12-2001
		ES2215618T T3	16-10-2004
		JP11029828 A	02-02-1999
		JP11034057 A	09-02-1999
		JP11035733 A	09-02-1999
		JP2000026295	25-01-2000
		JP2002540144T	26-11-2002
		NZ333023 A	25-08-2000
		NZ514767 A	27-02-2004
		PT1162980T T	30-06-2004
		SG89259 A1	18-06-2002
		TW397697 B	11-07-2000
		US6043259 A	28-03-2000
		US6066229 A	23-05-2000
		US6435249 B1	20-08-2002
WO0053606	14-09-2000	AU763464 B2	24-07-2003
		AU3183400 A	28-09-2000
		BR0008857 A	18-12-2001
		CA2366602 A1	14-09-2000
		EP1169322 A1	09-01-2002
		JP2002539127T	19-11-2002
		NZ514567 A	26-11-2002
		US6339085 B1	15-01-2002
		US2001031770 A 1	18-10-2001
		US2003195236 A1	16-10-2003
WO0164692	07-09-2001	AU3718501 A	12-09-2001
		CA2401655 A1	07-09-2001
		EP1268498 A1	02-01-2003
		JP2003525303T	26-08-2003
		US6605612 B2	12-08-2003
		US6667315 B2	23-12-2003
		US6780997 B2	24-08-2004
		US6867215 B2	15-03-2005
		US2003114677 A1	19-06-2003
		US2004171588 A1	02-09-2004